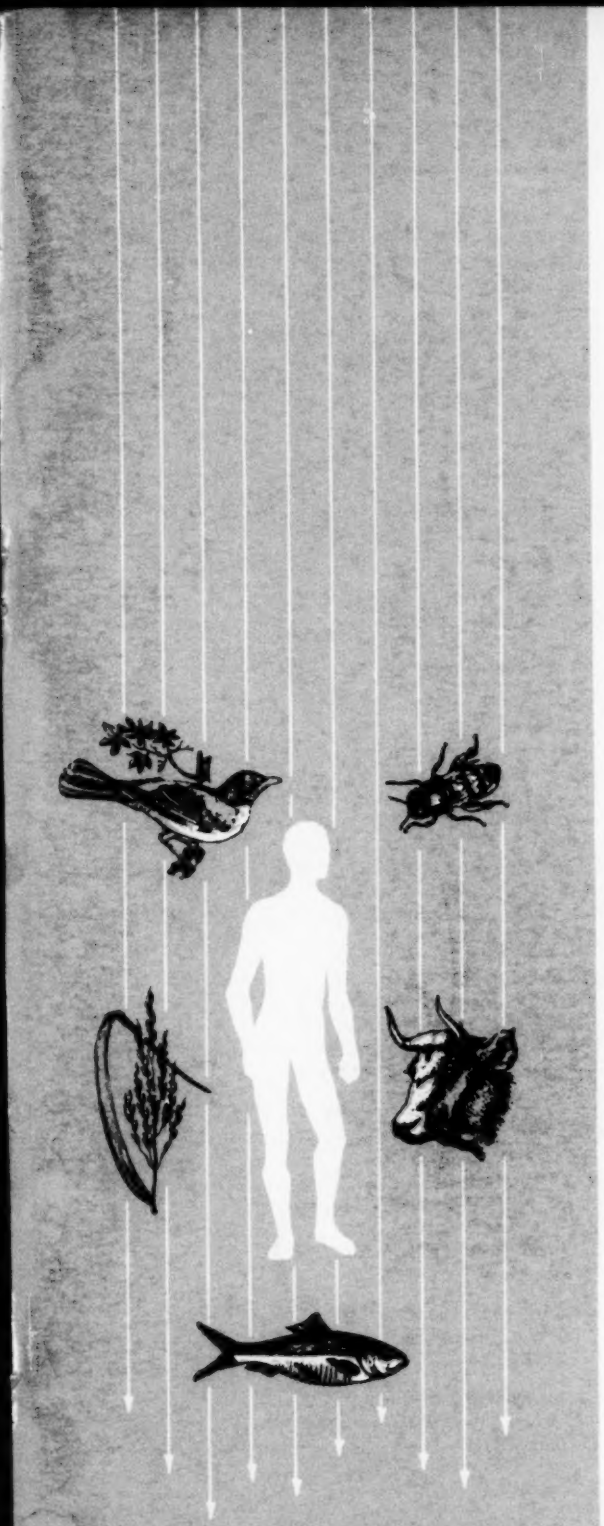

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EDITORIAL

New Publications from Monitoring Panel

Journal subscribers have recently received in the mail *Guidelines on Sampling and Statistical Methodologies for Ambient Pesticide Monitoring*. Newest publication of the Monitoring Panel of the Federal Working Group on Pest Management, the *Guidelines* represent an attempt to standardize sampling procedures among the various components of the National Pesticide Monitoring Program. Guideline compilers hope by this means to minimize variations encountered by personnel sampling various media in widely differing geographic regions.

For 10 years the Monitoring Panel has coordiated various Federal activities concerned with pesticide monitoring. The National Pesticide Monitoring Program, begun in 1964 as an interagency cooperative effort, is the prime result of this coordination. Other charter responsibilities of the Monitoring Panel include disseminating data obtained from pesticide monitoring activities, maintaining awareness of monitoring programs and plans of all Federal agencies, and developing manuals or guidelines to encourage acceptable sampling and analytical methodologies in pesticide monitoring.

Since its initial appearance in June 1967, the *Pesticides Monitoring Journal* has fulfilled the first responsibility mentioned above. Another charge of the charter inspired the *Catalog of Federal Pesticide Monitoring Activities*, originally published in 1968 and now being revised for publication in spring 1975. The new edition will cover activities in effect through June 1973, greatly enlarging its predecessor. The catalog is a reference source identifying types and locations of all Federal pesticide monitoring activities. The Panel is also putting final touches on its *Guidelines on Analytical Methodologies for Ambient Pesticide Monitoring* which is targeted for publication next summer.

A decade ago the Panel undertook to develop a continuing network to monitor pesticide residue levels in air, water, soil, wildlife, fish, and humans: an ambitious goal for six different agencies, each with different and sometimes divergent reasons for undertaking the task. Today pesticide residue levels are monitored on a national scale and resulting data are disseminated widely by countless scientists and authors from Federal, State, and local agencies, and academic and international sources, through the *Pesticides Monitoring Journal*.

This tradition of interagency cooperation is further reinforced by the catalog and two sets of guidelines. We trust that these three publications will prove invaluable to individuals and organizations concerned with pests, pesticides, and pest management.

Anthony Inglis
Assistant Executive Secretary

PESTICIDES IN PEOPLE

Organochlorine Insecticide Residues in Human Milk in Portugal[1]

Isabel Graca, A. M. S. Silva Fernandes, and H. C. Mourão

ABSTRACT

A total of 222 samples of human milk collected in several parts of Portugal were analyzed by gas chromatography in 1970 and 1972. The highest rate of insecticide contamination was observed in the 1970 study in a sample taken from an employee who for 6 years had been cleaning glass equipment used in the analysis of pesticide formulations. Her milk contained 1.38 ppm total DDT 73 days after her child had been delivered.

DDT, the most frequently detected insecticide, was found in 99.5 percent of the samples. Mean residues of total DDT were similar to those of other European and North American countries. Dieldrin was detected in 15 samples; values ranged from 0.005 to 0.031 ppm.

In the 1972 study, p,p'*-DDE residues ranged from 0.006 to 0.699 ppm and* p,p'*-DDT residues ranged from 0.003 to 0.345 ppm. The highest mean value of total DDT, 0.326 ± 0.175 ppm, was from the district of Lisbon.*

Potatoes, fruits, and vegetables were the most important items in the women's diets except in coastal districts where the staple was fish. Most of the participating women were homemakers.

Introduction

Widespread use of persistent pesticides has led to unintentional pollution of the environment; it is becoming increasingly difficult to find any being in inhabited regions of the globe which does not contain residues of DDT or its metabolites. Owing to its liposolubility, an inherent property of numerous organochlorine insecticides, DDT is readily absorbed by living organisms; it is stored in certain organs but accumulates primarily in the adipose tissues.

DDT is detected with greater frequency than are other organochlorines because it was widely used in agriculture and public health for so many years, but residues of other insecticides such as dieldrin, heptachlor epoxide, and BHC (known in European countries as HCH) are also fairly common.

In addition to organochlorines, other substances which have been detected recently are polychlorinated biphenyls (PCB's). Widely used in industry, PCB's are approximately as persistent and as liposoluble as are organochlorines. It may be concluded that they also cause considerable, if unintentional, contamination of the environment.

Because humankind is in direct contact with these products, unintentionally ingesting them in foodstuffs or intentionally using them as pesticides, it is not surprising that insecticide residues are found in human adipose tissues, blood, urine, and milk. Residue levels vary with the type of exposure to which the individual is subjected and the individual's daily intake of residues in foodstuffs. This accounts for the variations in levels observed in different professions, between rural and urban zones, between countries, and even between developed and underdeveloped areas.

The first research into pesticide residues in human milk was undertaken in 1950 by Laug et al. (*1*), who detected DDT in 30 of 32 samples examined. However, it was only upon introduction of organochlorine insecticide residue analysis by gas chromatography that these studies were intensified. To date, innumerable studies on this matter have been published in various countries.

In Portugal, no research into unintentional insecticide contamination was carried out before 1966. In that year, samples of cows' milk, butter, and cream were analyzed for the first time. Results disclosed the presence of DDT and its metabolites in all these dairy products. In subsequent years, studies were extended to embrace residues in wildlife. As expected, work undertaken to date has revealed widespread organochlorine pesticide contamination in Portugal.

[1] Direcção-Geral dos Servicos Agricolas, Laboratório de Fitofarmacologia, Oeiras, Portugal.

In 1970 a preliminary study of human milk entailing analysis of 54 samples was made. A sample was obtained from an employee of the Laboratório de Fitofarmacologia who assisted in analyses of pesticide formulations. Other samples were taken at two Lisbon maternity hospitals from lactating women whose case histories were unknown.

In light of the results of this preliminary study and the absence of details in the women's case histories, a more detailed study was undertaken which involved individuals from various parts of Portugal. The present report describes results of both the preliminary study and the 1972 study.

Analytical Methods

The amount of milk sampled varied among the individual women and ranged between 5 and 20 ml. Samples were refrigerated until analysis. Upon collection, 1 ml of a potassium dichromate saturated aqueous solution having 1 percent amyl alcohol was added to act as a preservative.

A method devised by de Faubert Maunder et al. (*2*) was used to extract and purify samples; the residues were determined by gas chromatography using an electron-capture detector. The method detected residues as low as 0.001 ppm for DDT isomers and metabolites, dieldrin, HCB, and BHC. Results were confirmed by using different column packings (10 percent DC 200, 6 percent QF_1 + 4 percent SE-30, and 5 percent QF_1 on 80/100 mesh Gas Chrom Q). In addition, one-fifth the samples were submitted to alkaline hydrolysis to confirm the DDT residues. Residue values given in the tables are not corrected for recovery, which ranged from 84 to 96 percent.

Fat content was determined by drying a subsample extracted with acetone and weighing the respective residue.

RESULTS OF 1970 PRELIMINARY STUDY

The first milk sample analyzed was obtained from an employee of the Laboratório de Fitofarmacologia who for 6 years had been cleaning glass equipment used in analyses of pesticide formulations. This woman had therefore been in daily contact with these products. Her milk was extracted at 73, 77, 85, 92, and 98 days after delivery of her child, who happened to be the firstborn. Results are summarized in Table 1.

All the remaining 53 samples collected at two Lisbon maternity hospitals contained residues of *p,p'*-DDE and *p,p'*-DDT. Dieldrin was found in four of the samples; *o,p'*-DDT was found in one. DDE residues ranged from 0.020 to 0.390 ppm and *p,p'*-DDT ranged from 0.008 to 0.136 ppm. Total DDT residues varied from 0.032 to 0.497 ppm; the mean was 0.177 and the standard deviation was ± 0.108 ppm. Dieldrin residues ranged from 0.018 to 0.031 ppm.

RESULTS OF 1972 STUDY

Authors collected and analyzed 168 milk samples from 12 districts in Portugal. Each sample was accompanied by a data sheet identifying the mother and containing relevant particulars such as date and number of deliveries, occupation, and normal intake of staple foods.

Results of the analyses are summarized in Tables 2-13. Of the 168 samples examined, 167 showed residues of *p,p'*-DDE and *p,p'*-DDT. Dieldrin was detected in four samples from the district of Bragança, one from Setúbal, and six from Viseu. In a negligible number of samples, residues of γ BHC and hexachlorobenzene were identified but were not quantified because levels were very low.

Mean values, standard deviations, and maximum and minimum residues of total DDT for each set of samples from the various districts are contained in Table 14. Converted means relating to total DDT were transformed by $\sqrt[4]{X}$ and are summarized in Table 15. This transformation was suggested by the detected correlation between the logarithm of the mean and the logarithm of the variance for each district (*3*).

Having established that Bragança was the district theoretically least polluted with DDT, authors determined the significance of the differences for all the remaining means (*4*). Highly significant differences of 1 percent were observed for Odivelas, Amadora, Loures, Caneças (all within the district of Lisbon); Portalegre; Aveiro; Evora; and Setúbal. Differences for Damaia (in the district of Lisbon), Guarda, and Porto were significant (5 percent). Differences for Faro, Vila Real, Viana do Castelo, and Viseu did not reach the level of significance.

Results of the inquiry into dietary habits of the lactating women revealed that in most cases potatoes, fruits, and vegetables were important items in their diets. Only a few women claimed to drink milk frequently and, in some districts, the number of nursing mothers who never drank it at all was very high. In the coastal districts the staple food was usually reported to be fish.

Table 16 indicates that most of the mothers were homemakers. Sample 6 from the district of Setúbal was obtained from a farmworker; sample 1 from Faro and sample 3 from Caneças were from factory workers. The mother from Faro was working in an almond-shelling factory; no details are available on the specific type of factory work being done by the woman from Caneças.

Discussion

References cited in this paper show that the pesticide most widely found in human milk is DDT and its metabolites, mainly DDE (*5-23*); TDE has been detected

in some cases (*6,10,18,22*). The *o,p'*-DDT isomer was detected by Ritcey et al. (*22*) and by Curley and Kimbrough (*6*). The latter authors also detected the *o,p'*-DDE isomer.

In the present study DDT was by far the most frequently detected insecticide; it was found in 99.5 percent of the samples analyzed. Both *p,p'*-DDT and *p,p'*-DDE were present in all samples containing DDT; residue levels of the latter usually were higher than those of the former. Juszkiewicz (*17*) and Ritcey et al. (*22*) arrived at similar conclusions.

The lactating woman who was employed by our Laboratório de Fitofarmacologia revealed the highest level of DDT, 1.38 ppm, 73 days after delivery. The closest value mentioned in the literature reviewed was reported by Gracheva (*8*), who detected 1 mg/liter total DDT.

Table 1 shows that the DDT level in milk decreased as the number of days between sampling and delivery increased.

TABLE 1. *DDT residues detected in milk of an employee of Laboratório de Fitofarmacologia, Oeiras, Portugal—1970*

Days Between Delivery and Sampling	Residues, ppm		Total DDT, ppm
	p,p'-DDE	*p,p'*-DDT	
73	0.938	0.444	1.38
77	0.680	0.292	0.97
85	0.850	0.350	1.20
92	0.408	0.140	0.55
98	0.263	0.097	0.36

TABLE 2. *Pesticide residues in human milk from district of Aveiro, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	2	172	0.66	ND	0.025—	0.022	0.047—	3.79	3.33	7.12
2	1	33	3.67	ND	0.123	0.042	0.165	3.35	1.14—	4.49
3	1	52	2.20	ND	0.063	0.039	0.102	2.86	1.75	4.61
4	1	143	4.48	ND	0.491+	0.205	0.696	10.95+	4.58	15.53+
5	2	171	3.21	ND	0.076	0.042	0.118	2.35	1.31	3.66
6	1	113	0.70	ND	0.117	0.091	0.208	1.57—	1.30	2.87—
7	5	240	4.86	ND	0.312	0.196	0.508	6.42	4.03	10.45
8	6	141	0.47	ND	0.030	0.019—	0.049	6.28	4.04	10.32
9	1	48	3.36	ND	0.116	0.073	0.189	3.45	2.17	5.62
10	1	78	5.67	ND	0.398	0.345+	0.743+	7.01	6.08+	13.09

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 3. *Pesticide residues in human milk from district of Bragança, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	2	113	6.15	ND	0.045	0.020	0.065	0.72	0.39—	1.11
2	6	46	2.24	ND	0.024	0.024	0.048	1.06	1.07	2.13
3	1	225	3.33	ND	0.041	0.016	0.057	1.24	0.48	1.72
4	3	31	2.01	ND	0.013	0.008	0.021	0.65	0.40	1.05
5	4	152	4.44	ND	0.039	0.031	0.070	0.89	0.70	1.59
6	1	37	2.82	ND	0.067	0.023	0.090	2.39+	0.82	3.21
7	4	33	2.55	0.013	0.022	0.016	0.038	0.87	0.63	1.50
8	2	44	3.46	ND	0.078	0.028	0.106	2.24	0.81	3.05
9	2	213	3.71	ND	0.053	0.045	0.098	1.42	1.21	2.63
10	1	326	7.84	ND	0.097+	0.052+	0.149+	1.24	0.66	1.90
11	4	157	1.29	ND	0.017	0.009	0.026	1.31	0.70	2.04
12	11	30	2.06	ND	0.026	0.020	0.046	1.28	0.97	2.25
13	10	186	2.53	ND	0.028	0.013	0.041	1.11	0.51	1.62
14	6	196	2.11	ND	0.020	0.019	0.039	0.95	0.90	1.85
15	8	284	4.21	0.006	0.025	0.021	0.046	0.58	0.50	1.08
16	2	31	2.84	0.013	0.063	0.035	0.098	2.22	1.23+	3.45+
17	5	287	5.86	0.009	0.025	0.029	0.054	0.64	0.75	1.39
18	3	67	2.91	ND	0.051	0.024	0.075	1.75	0.82	2.57
19	11	222	2.20	ND	0.006—	0.004—	0.010—	0.27—	0.18	0.45—
20	1	131	3.80	ND	0.066	0.016	0.082	1.74	0.42	2.16

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 4. *Pesticide residues in human milk from district of Évora, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	3	4	3.76	ND	0.224	0.081	0.305	5.94	2.15	8.09
2	1	3	1.58	ND	0.111	0.026	0.137	7.03	1.65	8.68
3	1	4	4.80	ND	0.198	0.079	0.277	4.13	1.65	5.78
4	2	14	1.70	ND	0.081	0.028	0.109	4.75	1.65	6.40
5	3	8	1.76	ND	0.019—	0.012—	0.031—	1.10—	0.68—	1.78—
6	1	22	2.62	ND	0.330	0.165+	0.495	12.60+	6.30+	18.90+
7	2	2	3.23	ND	0.156	0.064	0.220	4.83	1.98	6.81
8	2	10	7.62	ND	0.699+	0.138	0.837+	9.17	1.82	10.99
9	1	5	0.73	ND	0.046	0.017	0.063	6.30	2.33	8.63
10	1	3	1.68	ND	0.174	0.037	0.211	10.36	2.20	12.56

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 5. *Pesticide residues in human milk from district of Portalegre, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	4	43	2.50	ND	0.059	0.015—	0.074	2.34—	0.60	2.94—
2	1	28	3.24	ND	0.162	0.058	0.220	5.00	1.79	6.79
3	3	180	3.52	ND	0.239	0.066	0.305	6.79	1.88	8.67
4	2	139	1.39	ND	0.061	0.025	0.086	4.39	1.80	6.19
5	4	90	6.46	ND	0.333	0.273+	0.606+	5.15	4.23+	9.38
6	1	55	4.96	ND	0.221	0.029	0.250	4.45	0.58—	5.03
7	1	79	4.33	ND	0.364+	0.124	0.488	8.41+	2.86	11.27+
8	1	131	3.77	ND	0.090	0.068	0.158	2.37	1.80	4.17
9	1	150	3.94	ND	0.230	0.088	0.318	5.84	2.23	8.07
10	3	134	1.58	ND	0.039—	0.018	0.057—	2.44	1.14	3.58

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 6. *Pesticide residues in human milk from district of Faro, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	1	15	4.14	ND	0.216+	0.056+	0.272+	5.22	1.35	6.57
2	3	211	0.69	ND	0.025—	0.013—	0.038—	3.57—	1.88+	5.45—
3	1	45	1.28	ND	0.048	0.023	0.071	3.71	1.80	5.51
4	1	158	2.27	ND	0.128	0.029	0.157	5.64+	1.28—	6.92+
5	2	45	3.40	ND	0.138	0.052	0.190	4.06	1.53	5.59

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 7. *Pesticide residues in human milk from district of Guarda, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	1	9	2.07	ND	0.122	0.019—	0.141	5.88	0.92	6.80
2	1	240	5.66	ND	0.163	0.050	0.213	2.07	0.88	2.95
3	4	90	3.55	ND	0.048—	0.024	0.072—	1.34	0.68	2.02
4	4	31	5.56	ND	0.118	0.056	0.174	2.11	1.01	3.12
5	2	9	3.02	ND	0.229	0.096+	0.325	7.60+	3.18+	10.78+
6	1	35	3.38	ND	0.059	0.024	0.083	1.75	0.71	2.46
7	3	44	5.59	ND	0.056	0.019—	0.075	1.02—	0.34—	1.36—
8	1	40	5.70	ND	0.282+	0.084	0.366+	4.95	1.47	6.42
9	8	103	3.43	ND	0.050	0.053	0.103	1.46	1.55	3.01
10	1	121	3.86	ND	0.070	0.027	0.097	1.81	0.70	2.51

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 8. *Pesticide residues in human milk from district of Lisbon, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	1	23	3.84	ND	0.278	0.180	0.458	7.24	4.68	11.92
2	1	9	2.43	ND	0.353	0.099	0.452	14.53	4.06	18.59
3	1	30	2.00	ND	0.140	0.043	0.183	5.00	1.54	6.54
4	1	33	4.64	ND	0.410	0.134	0.544	8.84	2.89	11.73
1 a	1	38	6.49	ND	0.087	0.059	0.146	1.34—	0.91	2.25—
2 a	1	48	1.66	ND	0.158	0.040	0.198	9.52	2.41	11.93
3 a	1	324	5.37	ND	0.230	0.068	0.298	4.28	1.27	5.55
4 a	3	28	4.64	ND	0.185	0.081	0.266	3.99	1.75	5.74
5 a	2	198	6.06	ND	0.198	0.128	0.326	3.27	2.11	5.38
1 b	2	104	2.76	ND	0.180	0.051	0.231	6.52	1.85	8.37
2 b	1	23	3.35	ND	0.134	0.044	0.178	3.99	1.31	5.30
3 b	1	26	4.37	ND	0.152	0.073	0.225	3.48	1.67	5.15
4 b	2	24	6.41	ND	0.140	0.095	0.235	2.19	1.48	3.67
1 c	2	120	2.15	ND	0.054—	0.029—	0.083—	2.52	1.35	3.87
2 c	2	30	3.06	ND	0.129	0.056	0.185	4.22	1.83	6.05
3 c	1	99	3.95	ND	0.108	0.034	0.142	2.73	0.86—	3.39
4 c	1	36	1.93	ND	0.600+	0.180	0.780+	31.09+	8.70+	39.79+
5 c	3	52	2.15	ND	0.267	0.084	0.351	12.40	3.91	16.31
6 c	3	Unknown	0.98	ND	0.095	0.041	0.136	9.70	4.18	13.88
7 c	1	58	4.11	ND	0.268	0.186	0.454	6.53	4.53	11.06
8 c	1	182	4.36	ND	0.202	0.072	0.274	4.62	1.65	6.27
9 c	3	14	4.32	ND	0.138	0.089	0.227	3.20	2.06	5.26
10 c	2	182	6.16	ND	0.251	0.103	0.354	4.07	1.67	5.74
11 c	2	29	4.71	ND	0.180	0.064	0.244	3.82	1.36	5.18
1 d	1	33	4.52	ND	0.371	0.086	0.457	8.21	1.90	10.11
2 d	1	93	2.83	ND	0.177	0.065	0.242	6.25	2.30	8.55
3 d	3	156	4.81	ND	0.404	0.187	0.591	8.39	3.89	12.28
4 d	1	35	5.62	ND	0.357	0.340+	0.697	6.35	6.05+	12.40
5 d	2	50	5.52	ND	0.315	0.189	0.504	9.98	5.35	15.33

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 9. *Pesticide residues in human milk from district of Porto, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	6	9	1.89	ND	0.089	0.048	0.137	4.71	2.52+	7.23
2	1	65	6.60	ND	0.248+	0.105+	0.353+	3.75	1.59	5.34
3	4	24	4.05	ND	0.075	0.028	0.103	1.84	0.69—	2.53
4	3	114	2.41	ND	0.094	0.025	0.119	3.90	1.04	4.94
5	2	360	2.62	ND	0.077	0.023	0.100	2.91	0.88	3.79
6	3	234	1.92	ND	0.025	0.019	0.044	1.28	0.99	2.27
7	1	57	2.04	ND	0.032	0.028	0.060	1.55	1.37	2.92
8	1	33	2.05	ND	0.031	0.015	0.046	1.51	0.73	2.24
9	2	29	3.91	ND	0.237	0.071	0.308	6.06+	1.82	7.88+
9a	Unknown	89	1.97	ND	0.068	0.032	0.100	3.43	1.62	5.05
10	Unknown	77	3.92	ND	0.145	0.057	0.202	3.70	1.44	5.14
11	Unknown	63	3.54	ND	0.104	0.045	0.149	2.94	1.27	4.21
12	Unknown	32	1.83	ND	0.040	0.016	0.056	2.23	0.87	3.10
13	Unknown	47	2.53	ND	0.059	0.028	0.087	2.33	1.11	3.44
14	1	211	4.37	ND	0.162	0.036	0.198	3.71	0.82	4.53
15	2	65	4.40	ND	0.200	0.066	0.266	4.55	1.50	6.05
16	6	174	2.21	ND	0.022—	0.048	0.070	0.93—	2.17	3.15
17	1	328	2.02	ND	0.026	0.015—	0.041—	1.29	0.74	2.03—
18	1	176	1.94	ND	0.059	0.027	0.086	3.04	1.39	4.43
19	4	69	4.08	ND	0.084	0.071	0.155	2.06	1.74	3.80
20	1	60	2.78	ND	0.127	0.045	0.172	4.57	1.62	6.19

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 10. *Pesticide residues in human milk from district of Setúbal, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	DDT Total
1	Unknown	56	0.41	ND	0.031—	0.014—	0.045—	7.56	3.41	10.97
2	Unknown	Unknown	1.98	ND	0.067	0.035	0.102	3.38	1.77	5.15
3	Unknown	8	2.27	ND	0.309	0.176	0.485	13.61	7.75+	21.36+
4	Unknown	238	5.27	ND	0.166	0.130	0.296	3.14—	2.47	5.61
5	Unknown	124	5.51	ND	0.220	0.085	0.305	3.99	1.54	5.53
6	Unknown	28	4.83	ND	0.403	0.183+	0.586	8.34	3.79	12.13
7	5	11	1.38	ND	0.058	0.028	0.086	4.17	2.03	6.20
8	1	17	2.51	ND	0.087	0.047	0.134	3.48	1.87	5.35
9	2	186	2.02	ND	0.066	0.029	0.095	3.27	1.44	4.71—
11	1	210	2.57	ND	0.190	0.075	0.265	7.40	2.92	10.32
12	2	63	5.93	ND	0.229	0.076	0.305	3.86	1.28—	5.14
13	4	68	2.70	ND	0.112	0.061	0.173	4.13	2.26	6.39
14	3	164	1.17	0.006	0.038	0.024	0.062	3.28	2.05	5.33
15	1	40	2.97	ND	0.516+	0.113	0.629+	17.37+	3.80	21.17

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 11. *Pesticide residues in human milk from district of Viana do Castelo, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	1	61	2.34	ND	0.072	0.031	0.103	3.09	1.32	4.41
2	2	50	2.75	ND	0.076	0.053	0.129	2.76	1.93	4.69
3	1	5	0.40	ND	0.020	0.019	0.039	5.00	4.75+	9.75+
4	2	178	1.94	ND	0.123	0.048	0.171	6.44+	2.47	8.91
5	1	43	5.08	ND	0.146+	0.113+	0.259+	2.87	2.22	5.09
6	2	18	1.52	ND	0.042	0.015	0.057	2.74	0.99	3.73
7	2	324	3.89	ND	0.083	0.047	0.130	2.14	1.21	3.35
8	2	232	1.58	ND	0.019	0.010	0.029	1.17—	0.63—	1.80—
10	3	110	0.45	ND	0.007—	0.003—	0.010—	1.56	0.67	2.23

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 12. *Pesticide residues in human milk from district of Vila Real, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	*p,p'*-DDE	*p,p'*-DDT	Total DDT
1	3	129	2.02	ND	0.043	0.020	0.063	2.11	0.99	3.10
2	1	159	3.97	ND	0.100	0.047	0.147	2.52	1.18	3.70
3	9	78	1.31	ND	0.025—	0.010—	0.035—	1.87	0.76—	2.63
4	10	132	1.27	ND	0.043	0.024	0.067	3.41	1.89	5.30
5	1	41	4.01	ND	0.155	0.058+	0.213	3.86	1.44	5.30
6	1	16	1.69	ND	0.107	0.044	0.151	6.33+	2.60	8.93+
7	4	62	3.94	ND	0.065	0.033	0.098	1.66—	0.84	2.50—
8	1	20	3.99	ND	0.204+	0.048	0.252+	5.11	1.20	6.31
9	4	30	1.32	ND	0.054	0.055	0.109	4.06	4.17+	8.23

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

TABLE 13. *Pesticide residues in human milk from district of Viseu, Portugal—1972*

Sample	Number of Deliveries	Days Between Delivery and Sampling	Fat Content, %	On Whole-Milk Basis, ppm				On Fat Basis, ppm		
				Dieldrin	p,p'-DDE	p,p'-DDT	Total DDT	p,p'-DDE	p,p'-DDT	Total DDT
1	3	65	1.67	ND	0.066	0.042	0.108	3.92	2.51	6.43
2	4	88	1.85	ND	0.021	0.018	0.039	1.14	0.97	2.11
3	4	208	1.40	ND	0.015	0.011	0.026	1.04	0.79	1.83
5	1	18	1.91	ND	0.036	0.012	0.048	1.86	0.63	2.49
6	1	17	3.42	0.009	0.057	0.057	0.114	1.67	1.67	3.34
7	4	193	3.71	0.005	0.008	0.009	0.017	0.22—	0.24—	0.46—
8	1	90	1.05	ND	0.027	0.013	0.040	2.57	1.24	3.81
9	2	128	3.63	0.017	0.032	0.016	0.048	0.87	0.44	1.31
11	1	164	2.19	ND	0.044	0.022	0.066	1.99	1.00	2.99
12	1	218	1.84	0.015	0.021	0.016	0.037	1.13	0.85	1.98
13	6	164	0.80	0.005	0.006—	0.004—	0.010	0.62	0.44	1.06
14	2	83	1.81	ND	0.020	0.020	0.040	1.10	1.11	2.21
15	2	18	2.75	ND	0.109+	0.158+	0.267+	3.97	5.73+	9.70+
16	2	93	0.96	ND	0.041	0.014	0.055	4.22+	1.46	5.68
17	1	42	3.19	ND	0.047	0.025	0.072	1.47	0.78	2.25
18	3	69	2.54	0.021	ND	ND	0.01—	ND	ND	—
19	1	20	1.49	ND	0.029	0.028	0.057	1.92	1.88	3.80
20	5	22	2.12	ND	0.018	0.011	0.029	0.83	0.52	1.35

NOTE: ND = not detected.
— = lowest value.
+ = highest value.

Tables 14 and 15 show that lowest mean levels of DDT were found in the rural districts Bragança and Viseu. The highest mean level was found in the district of Lisbon. Various other authors have reported lower contamination levels in rural areas than in urban areas (*16,18,21*).

Kroger (*12*) observed that mothers nursing firstborn children showed higher levels of DDT than did other mothers. This may account for the difference observed between mean levels in nursing mothers in the district of Lisbon and those in Bragança, but could hardly justify the difference in relation to Viseu. However, it is not only the number of deliveries that influences an individual's contamination levels, but also the number of days separating sampling from delivery, and the individual's dietary habits. Authors have not drawn conclusions regarding the influence of each of these factors.

Dieldrin was detected in 15 of 222 samples examined; levels ranged from 0.006 to 0.031 ppm. The district of Viseu showed the highest percentage of samples containing this insecticide; it was found in 33 percent of the samples collected in that area. In Bragança, 20 percent of the samples had dieldrin residues. Similar dieldrin levels have been reported by various authors (*6,7,10,13,16,19-22*). Most significant is the Japanese study in which residues of this insecticide were found in 74.6 percent of 398 samples of human milk (*16*). In Australia, Newton observed that in 67 samples examined, about 43 percent contained dieldrin residues (*18*).

Apart from DDT and dieldrin, BHC isomers are the pesticides which have been most widely reported in human milk. In the present case, γ BHC was detected in some samples but, because of its low level, was not measured. This was the only isomer reported by Westoo et al. in human milk in Sweden (*10*) but the β isomer has been detected at very high levels by various authors (*13,16,19-21*).

TABLE 14. *Total DDT residues in human whole milk, Portugal—1972*

District	Residues, mg/kg			Standard Deviation	Average Number of Deliveries
	Minimum	Maximum	Mean		
Aveiro	0.047	0.743	0.283	±0.265	2.1
Bragança	0.010	0.149	0.063	±0.034	4.4
Évora	0.031	0.837	0.269	±0.241	1.7
Faro	0.038	0.272	0.146	±0.094	1.6
Guarda	0.072	0.366	0.165	±0.106	2.6
Lisbon	0.083	0.780	0.326	±0.175	1.6
Porto	0.041	0.353	0.136	±0.088	Unknown
Portalegre	0.057	0.606	0.256	±0.181	2.1
Setúbal	0.032	0.629	0.252	±0.197	Unknown
Vila Real	0.035	0.252	0.126	±0.072	3.8
Viana do Castelo	0.010	0.259	0.103	±0.079	1.8
Viseu	<0.010	0.343	0.064	±0.075	2.4

TABLE 15. *Converted mean of total DDT residues in human whole milk submitted to the $\sqrt[4]{x}$ transformation, Portugal—1972*

County or District	Converted Mean, mg/kg	Significance Level, % [1]
Odivelas [2]	0.4778	1
Amadora [2]	0.3831	1
Loures [2]	0.2570	1
Caneças [2]	0.2396	1
Damaia [2]	0.2163	5
Portalegre	0.2122	1
Aveiro	0.2067	1
Évora	0.2051	1
Setúbal	0.1962	1
Guarda	0.1453	5
Faro	0.1243	NS
Porto	0.1178	5
Vila Real	0.1121	NS
Viana do Castelo	0.0798	NS
Viseu	0.0415	NS
Bragança	0.0564	

NOTE: NS = not significant.
[1] Significance levels determined by the Dunnet test (Dunnet, 1964).
[2] Towns in the district of Lisbon.

In the present study of human milk in Portugal, samples also contained traces of HCB which were not measured. HCB was detected in human milk in Australia (*18*) and in France (*13,20*).

TABLE 16. *Professions of lactating women whose milk was analyzed, Portugal—1972*

District	Number of Mothers Questioned	Homemakers	Civil Servants	Factory Workers	Farmworkers	Other [1]
Aveiro	10	9				1
Bragança	19	19				
Evora	10	8	1			1
Faro	5	4		1		
Guarda	10	9				1
Lisbon	29	25	1	1		2
Porto	22	20	2			
Portalegre	10	9	1			
Setúbal	15	14			1	
Vila Real	10	8	1			1
Viano do Castelo	10	10				
Viseu	20	19	1			

[1] Dressmakers, office assistants, and shop assistants.

Some authors have also detected lindane (*7,10,22,23*), heptachlor epoxide (*6,7,13,20,22,23*), endrin (*16*), aldrin (*16*), and PCB's (*10,19*), but none of these products was detected in human milk in Portugal. PCB's were not even sought during this work.

Comparing mean residues of total DDT detected in each of the districts of Portugal with values published in other countries and tabulated in a paper presented by Ritcey et al. (*22*), values found in Portugal are all higher than those in the Netherlands (0.048 ppm) and are all lower than those published in works on Romania (0.530 ppm) and Poland (0.40 ppm). It may be concluded that mean residues of total DDT in Portugal were generally similar to those of other European and North American countries.

The sale of DDT, BHC, and heptachlor was forbidden in Portugal after January 1, 1974, and the use of aldrin, dieldrin, and endrin has been greatly restricted. It is hoped that the problem of unintentional contamination by organochlorine pesticides in our country will begin to right itself, however slowly.

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PESTICIDES IN WATER

A Study of the Distribution of Polychlorinated Biphenyls in the Aquatic Environment[1]

Hans J. Crump-Wiesner,[2] Herman R. Feltz,[3] and Marvin L. Yates[4]

ABSTRACT

Data gathered from monitoring activities and project studies indicate the ubiquitous occurrence and distribution of polychlorinated biphenyls (PCB's) in the aquatic environment. By 1972 residues had been detected in samples from 19 States representing nearly every region of the country. These findings permit a preliminary assessment of PCB contamination across the Nation: concentrations ranged from 0.1 to 4.0 μg/liter in unfiltered water samples and from 5.0 to 3,200 μg/kg in bottom sediments. PCB residues were also found in fish and aquatic plants. Samples were prepared by the same techniques used for general chlorinated insecticide detection, with special attention to cleanup and separation of PCB's from other compounds. Basic identification and quantification were made by dual-column electron-capture/gas-liquid chromatography and confirmed by gas-liquid chromatography/mass spectrometry whenever possible. In sediment samples from a south Florida drainage ditch, polychlorinated naphthalenes (PCN's) were observed. This is possibly the first evidence of PCN's in an environmental sample and illustrates the importance of developing analytical capability for the surveillance of other organochlorine compounds that may behave like chlorinated hydrocarbon pesticides.

The sampling program is broadening geographically and gradually increasing to more adequately define the distribution of PCB residues in major drainage basins of the United States.

[1] Presented to the Division of Pesticide Chemistry, 164th National Meeting of the American Chemical Society, New York, N.Y., August 29, 1972. Subsequently published in the *Journal of Research of the U.S. Geological Survey*, Vol. 1, No. 5. Reprinted here with permission because data are considered significant to readers of the *Pesticides Monitoring Journal*.

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Introduction

Within the past few years, polychlorinated biphenyls (PCB's) have been identified as a major environmental contaminant. Their detection has caused widespread concern and has generated intense interest in data relating to the presence and effects of these compounds, particularly in the aquatic environment. First produced about 40 years ago, PCB compounds have become increasingly useful in such industrial applications as components in transformers and capacitors, heat exchangers, paints, inks, dyes, and dust control agents.

Although much attention has been focused on estimating levels and potential hazards of PCB's in aquatic organisms, few data are available on the occurrence of PCB's in water and bottom sediments. The Geological Survey, U.S. Department of Interior, through its water-quality-monitoring activities and water-resources-assessment projects, has been alert to the PCB problem since it was first reported by Widmark in 1967 (*1*). Although the Geological Survey has no nationwide PCB assessment program, sufficient data have accumulated from pesticide residue programs to permit a preliminary assessment of PCB contamination of the Nation's hydrologic environment. This paper presents data gathered from these activities, showing the widespread occurrence of PCB's in significant concentrations in unfiltered surface water and ground water, bottom sediments, flora, and fauna.

Analytical Techniques

PCB residues were analyzed by the multiple-pesticide-residue methods for water, suspended sediment, and bottom material described by Goerlitz and Brown (*2*). Analytical procedures are appropriate for chlorinated pesticides as well as the general class of organochlorine

compounds. Special attention was given to cleanup and separation of PCB's from coextractives.

EXTRACTION OF WATER, SEDIMENT, AND BIOTA

One-liter unfiltered water samples were collected in precleaned glass bottles and extracted three times with hexane. The hexane portions were combined, dried with anhydrous Na_2SO_4, and concentrated to 1 ml before cleanup and analysis by electron-capture/gas-liquid chromatography (EC/GLC).

Fifty-gram sediment samples (dry-weight basis) were extracted with an acetone-hexane solvent. The sediment was dispersed first in acetone, and hexane was added to recover the acetone and the desorbed material. The extract was washed with distilled water, dried over Na_2SO_4, and concentrated to 5 ml for cleanup before EC/GLC analysis.

The two extraction procedures used for biota are described in an analytical manual issued by the Food and Drug Administration, U.S. Department of Health, Education, and Welfare (*3*). Fish samples were extracted with petroleum ether in a blender; aquatic plants were extracted with acetonitrile. The chlorinated hydrocarbon fraction was partitioned between petroleum ether and acetonitrile, dried, and concentrated to a final volume of 5 ml.

SEPARATION

Bottom sediment, fish, and plant extracts were cleaned following the Law and Goerlitz technique (*4*) requiring less time and smaller volumes of solvents for elution than do other widely used methods. Liquid/solid column chromatography was employed using two different types of semimicro columns in sequence (Fig. 1). Hexane extracts were first passed through an alumina column, and one fraction was further chromatographed on silica gel to separate PCB's from chlorinated insecticides. Successive chromatography on alumina and silica gel results in a simultaneous cleanup and separation of PCB's from the common insecticides, except aldrin, and a slight overlap of *p,p'*-DDE. To achieve a reduction in background interference, mercury was added to remove sulfur from bottom sediment extracts before they were applied to the silica column.

In order to insure reproducible chromatographic conditions, the activity of the adsorbents was carefully controlled. Water extracts were cleaned on a deactivated alumina microcolumn (*5*). When PCB's were detected in the cleaned water extracts, they were also separated on a semimicro silica gel column.

IDENTIFICATION

Basic identification was made by dual-column EC/GLC (DC-200 and QF-1/OV-17) and confirmed by gas-liquid chromatography/mass spectrometry (GLC/MS) when sample size and concentrations were sufficient. The amount of PCB's was determined by matching the unknown peaks on the chromatogram to the nearest commercial formulation and measuring the areas of four corresponding peaks. Retention time and peak area measurements were made with a digital electronic integrator. The lower detection limit for PCB residues was 0.1 μg/liter in water and 5.0 μg/kg in bottom sediment. Reported levels are subject to considerable error because of the complexity of multiple peaks, some peak alteration, and the occasional presence of mixtures of PCB's in environmental samples. At best, reported values are estimates that may be as much as 50 percent in error.

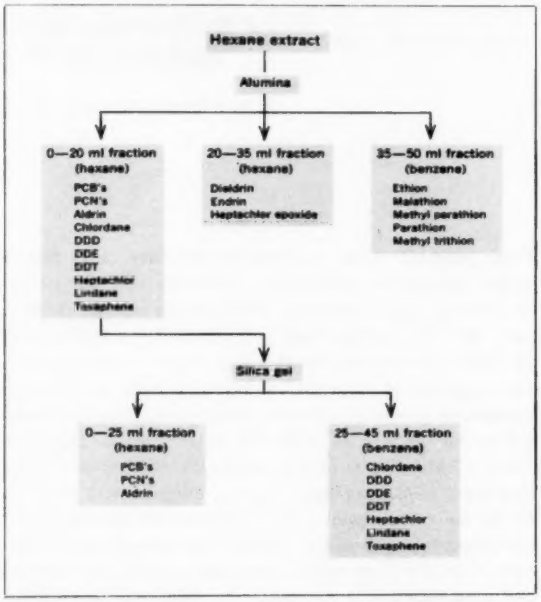

FIGURE 1. *Scheme for separating polychlorinated biphenyls (PCB's) and polychlorinated naphthalenes (PCN's) from pesticides*

DATA DESCRIPTION

Occurrences of pesticide residues in the aquatic environment have been documented over a period of years through monitoring programs of several Federal agencies. As early as 1957; studies of chlorinated hydrocarbon pesticides in major river basins were made by the Federal Water Pollution Control Administration, now a part of the U.S. Environmental Protection Agency, by use of the carbon-adsorption technique (*6*). In 1964 interagency cooperation in pesticide monitoring programs culminated in a proposal to begin a national monitoring program. The original program for water was described in 1967 in the first issue of the *Pesticides Monitoring Journal* (*7*). The purpose of this program, which was revised in 1971 (*8*), is to provide continuing information on levels of pesticide residues in the water resources of the Nation and to identify possible problem areas. Because PCB's are analyzed by the

multiple-pesticide residue techniques, routine reporting of these compounds has been incorporated into current pesticide programs. At present, samples from 20 of the 161 proposed network sites are collected and analyzed by the Geological Survey. All samples are collected from sites west of the Mississippi River and provide continuity with a network established to evaluate the quality of water used for irrigation (*9,10*). Budgetary restrictions have prevented further implementation of the network.

After development of the technique to separate PCB's from pesticide residues, examination for the presence of PCB's in water and suspended and bottom sediment samples collected for the National Monitoring Program began in January 1971. Funding was requested to increase the number of network stations to 50 in 1973, and to 100 in 1974, allowing a better assessment of PCB's and pesticides in major drainage basins throughout the United States. Analysis of 194 water samples and 33 bottom sediment samples revealed no positive identifications of PCB's; however, these data are not truly representative of the entire Nation because of the limited number of sites sampled.

In 1958, the Geological Survey began operation of a bench-mark network to provide basic hydrologic data on selected stream basins throughout the United States that are expected to remain in their present natural condition or are not expected to be significantly altered by humans. Locations of the 57 bench marks established in 37 States are shown in Figure 2.

To insure minimum interference by humans, many of the hydrologic bench marks are in national parks, wilderness areas, State parks, national forests, and areas set aside for scientific study. A detailed description of the network basins, including drainage, climate, topography, geology, vegetation, hydrology, water quality, and personmade influences, appears in a report by Cobb and Biesecker (*11*). Data gathered from 46 of these sites are presented in Table 1. Despite the careful screening for pristine location of bench-mark sites, two bottom sediment samples analyzed in the 1972 water year contained PCB residues. A value of 5.2 µg/kg was measured in the sample from South Fork Rocky Creek near Briggs, Tex., and 8.8 µg/kg was found in the sample from Upper Twin Creek at McGaw, Ohio.

The majority of the Geological Survey water resources studies are conducted in cooperation with State water resources and pollution control agencies, or in response to requests from other Federal agencies. PCB data from these programs in 35 States are presented in Tables 2-4.

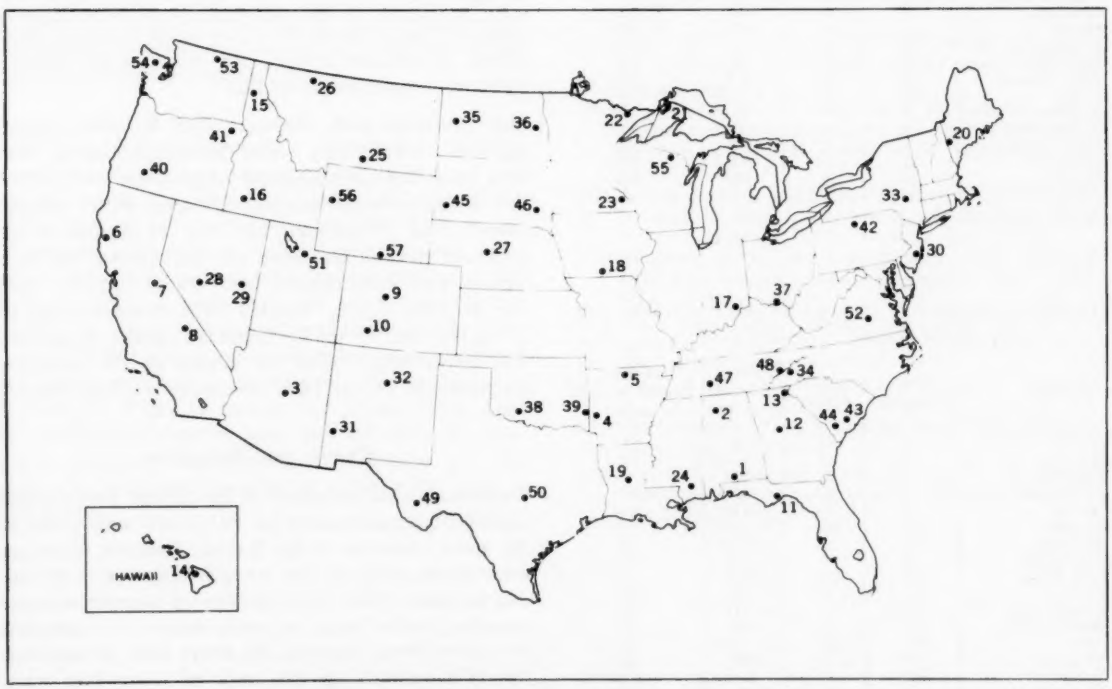

FIGURE 2. *Map showing hydrologic bench-mark stations.*
(Numbers refer to list in Cobb and Biesecker: refer to Literature Cited, reference 1.)

TABLE 1. *Summary of PCB residue data, national hydrologic bench-mark network, January 1971-June 1972*

Type of Sample	Unit	No. Samples	Occurrences	Concentration
Water	µg/liter	54	0	
Bottom sediment	µg/kg	51	2	5.2, 8.8

TABLE 2. *Summary of PCB residue data for surface and ground water, January 1971-June 1972*

State	No. Samples	Occurrences	Concentration, µg/liter	Median Concentration, µg/liter
Alaska	3	0	ND	ND
Arizona	8	0	ND	ND
Arkansas	32	0	ND	ND
California	161	2	0.1, 0.1	ND
Colorado	32	1	0.3	ND
Connecticut	13	6	0.1-0.2	0.1
Hawaii	5	0	ND	ND
Iowa	24	0	ND	ND
Kansas	10	0	ND	ND
Kentucky	7	0	ND	ND
Louisiana	9	0	ND	ND
Maine	2	0	ND	ND
Maryland	6	1	0.1	ND
Massachusetts	5	1	0.2	ND
Michigan	2	0	ND	ND
Minnesota	3	2	0.1, 0.3	ND
Mississippi	8	0	ND	ND
Missouri	21	0	ND	ND
Montana	47	0	ND	ND
Nebraska	44	0	ND	ND
New Jersey	11	3	0.1	0.1
New Mexico	36	0	ND	ND
New York	325	52	0.1-4.0	0.3
North Dakota	40	0	ND	ND
Oklahoma	19	0	ND	ND
Oregon	13	0	ND	ND
Pennsylvania	2	1	0.2	ND
Puerto Rico	7	1	0.1	ND
South Dakota	18	0	ND	ND
Texas	660	12	0.1-3.0	0.4
Virginia	4	1	0.1	ND
Washington	25	0	ND	ND
West Virginia	4	0	ND	ND
Wisconsin	3	0	ND	ND
Wyoming	18	0	ND	ND

NOTE: ND = not detected.

TABLE 3. *Summary of PCB residue data for bottom sediments, January 1971-June 1972*

State	No. Samples	Occurrences	Concentration, µg/kg	Median Concentration, µg/kg
Alaska	3	0	ND	ND
Arkansas	3	4	20-2,400	60
California	13	3	20-190	85
Connecticut	1	1	40	ND
Hawaii	4	0	ND	ND
Georgia	12	10	10-1,300	300
Maryland	11	5	10-1,200	30
Mississippi	8	2	50;170	ND
New Jersey	12	10	8-250	20
Oregon	4	2	15;140	ND
Pennsylvania	16	11	10-50	20
South Carolina	11	8	30-200	50
Texas	293	23	7.9-290	80
Virginia	10	8	5-80	40
Washington	10	0	ND	ND
West Virginia	2	1	10	ND

NOTE: ND = not detected.

TABLE 4. *Summary of PCB residue data from selected sampling sites in Florida, January 1971-June 1972*

Type Sample	No. Samples	Occurrences	Concentration, Range [1]	Median Concentration [1]
Water	231	12	0.1-2.1	0.2
Bottom sediment	118	50	5-3,200	30
Flora	16	5	10-50	20
Fauna	43	36	6-1,000	40

[1] Unit of measure: µg/liter for water samples, µg/kg for other samples.

Water samples alone, because of the low water solubility of PCB's, are not a good indicator of the widespread occurrence of the compounds, and show an incidence of slightly over 5 percent. Unfiltered water samples from 12 of the 35 States had PCB concentrations ranging from 0.1 to 4.0 µg/liter. However, a significant number of suspected traces of PCB's were not reported because of several analytical limitations. Generally, resampling of areas where PCB's were first detected revealed that the compounds were still present several months later.

Bottom sediments were collected concurrently with many of the water samples reported in Table 2. These samples were taken from lakes and streams that drain a variety of land-use areas generally located away from industrial centers. Data in Table 3 show that bottom sediment samples may be used as an indicator of PCB contamination in the Nation's hydrologic environment. Significantly, of samples collected at random from 16 States, 13 contained PCB's in the range of 5.0 to 2,400 µg/kg. Across the Nation, one of every five bottom sediment samples contained PCB's.

Data available from Florida (Table 4) merit special attention because they reveal the distribution of residues in several environmental components. Only 12 of 231 unfiltered water samples contained PCB's ranging from 0.1 to 2.1 µg/liter, but over 40 percent of the associated bottom sediments analyzed during the same period were contaminated with PCB's ranging from 5.0 to 3,200 µg/kg. Median PCB concentrations of 20 µg/kg and 40 µg/kg found in aquatic plants and fish, respectively, follow the scheme of the biological accumulation of the DDT family in southern Florida (*12*).

Results and Discussion

Preliminary data presented in this report indicate that significant concentrations of PCB's are widespread in the water resources of the Nation. However, there are some shortcomings of data compiled from pesticide residue programs. First is the problem of nonrepresentative sampling within States and some repetition of sampling in a given basin. Second, the lower limit of detection for PCB residues on the basis of a one-liter water sample is inadequate for critical evaluation. Trace amounts of less than 0.1 µg/liter were detected in a

significant number of samples but were excluded from the tabulation because they could not be confirmed. Third, because of the low solubility of PCB's in water, especially the higher chlorinated ones, the bulk of PCB residues in streams are associated with suspended sediment and bottom material. Therefore, PCB concentrations may be expected to vary directly with the suspended sediment concentration in the cross section of a stream. Most surface water samples were collected by depth integration at the center of flow, which usually does not provide a representative sample of suspended sediment. Future investigations should be made with a depth-integrating sampler using the equal-transit-rate procedure (*12*). In spite of these shortcomings, evidence for the ubiquity of PCB's in the hydrologic environment is clearly established.

As others have pointed out (*13*), polychlorinated naphthalenes (PCN's) are compounds that have uses similar to PCB's and may possibly be present in environmental samples. They can be separated from chlorinated hydrocarbon insecticides and are eluted in the same fraction as PCB's by alumina/silica gel column chromatography. Sediment samples collected from a south Florida drainage ditch contained mixtures of PCN's ranging from 1,250 to 5,000 μg/kg, whereas water samples overlying the sediments averaged 5.7 μg/liter. Identification was confirmed by both microcoulometry and GLC/MS. This is possibly the first evidence of PCN's in an environmental sample and illustrates the importance of developing analytical capability for the surveillance of other organochlorine compounds that may behave like chlorinated hydrocarbon pesticides.

It is probable that PCB's enter the aquatic environment through low-temperature incineration of solid wastes, industrial waste disposal into waterways, and sewage outfalls. The highest levels are usually associated with industrial areas and nearby aquatic food chains. In contrast, authors have noticed significant concentrations in the bottom sediments of drainage ditches, multipurpose canals, and suburban real estate lakes remote from major industrial and metropolitan areas. The presence of PCB's in real estate lakes has been attributed to a variety of construction materials used in the housing industry. PCB's in surface and ground water used for public water supplies were detected through cooperative programs. The highest concentration was 4.0 μg/liter in an untreated source for a city in the State of New York.

It is clear that the presence of PCB's and other organochlorine compounds in the aquatic environment merits continuing observation because of the limited evaluation that can be made from the meager data available. It is important to measure baseline levels of PCB's in streams and lakes in order to determine trends. Long-term monitoring on a systematic basis will provide the data necessary to assess the presence of PCB residues and concurrently reveal problem areas.

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RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Chlorinated Hydrocarbon Pesticide Residues in Oysters (Crassostrea commercialis) *in Moreton Bay, Queensland, Australia, 1970-72* [1]

Donald Edward Clegg

ABSTRACT

A 2-year survey was carried out to monitor levels of DDT, DDD, DDE, and dieldrin in oysters (Crassostrea commercialis) *in Moreton Bay, Queensland, Australia. Samples were taken at quarterly intervals from eight stations located at or near the mouths of streams entering the bay.*

Highest levels of 0.94 ppm DDT, 0.51 ppm DDD, 0.20 ppm DDE, and 0.34 ppm dieldrin were found in July 1970 at the sampling station on the Brisbane River 16 km downstream from the center of Brisbane, a city of 700,000. Maximum values at other stations were substantially lower.

Residue levels varied considerably throughout the 2-year period. Authors attribute this at least in part to seasonal rainfall patterns in the catchment areas.

Introduction

A program to monitor chlorinated hydrocarbon pesticide residues in oysters (*Crassostrea commercialis*) in Moreton Bay, Queensland, Australia, was begun in April 1970 and continued approximately at quarterly intervals until March 1972. The oyster was chosen as an indicator organism partly because of its ubiquity and immobility but mainly because Butler et al. have shown that it has a marked ability to concentrate certain chlorinated hydrocarbons from the surrounding water and, when subsequently placed in clean water, is able to purge itself rapidly of these compounds (*1*). These characteristics are useful not only in helping to determine geographical distribution of pesticides in this region but also in facilitating observation of seasonal variations of pesticide levels, particularly as a result of changes in the flow volume of fresh water in adjacent streams.

Moreton Bay is formed by two large sandy islands, Moreton and Stradbroke. The bay is fed by several rivers, the chief being the Brisbane River, and a number of smaller streams. The southern end is estuarine and the north coast faces relatively open water (Fig. 1).

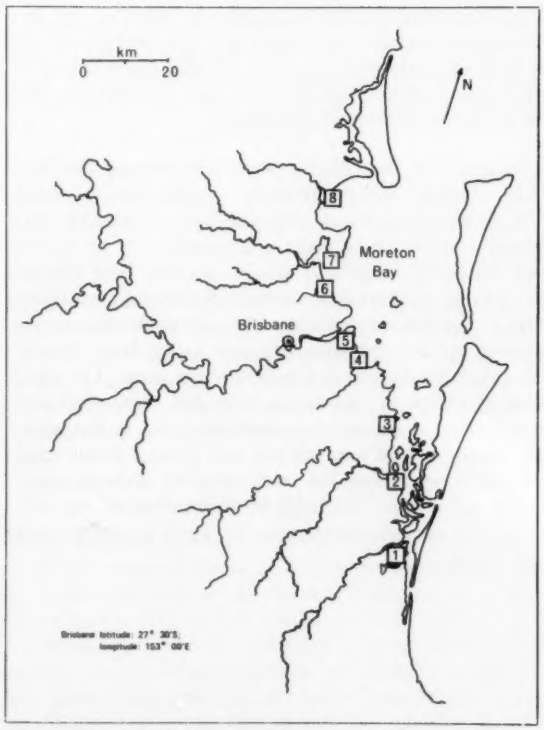

FIGURE 1. *Map of Moreton Bay area, Queensland, Australia, surveyed for pesticide contamination in oysters, 1970-72*

[1] Animal Research Institute, Department of Primary Industries, Queensland, Australia.

The city and suburbs of Brisbane occupy much of the central region of Moreton Bay, extending about 24 km inland. Industry is concentrated along the banks of the Brisbane River, especially toward the mouth. Smaller outlying towns, such as Caboolture, Cleveland, and Beenleigh, are centers for fruit, vegetable, sugar cane, and dairy farming; they also serve as dormitory towns for city commuters. The bay waters yield commercial quantities of fish, prawns, crabs, and oysters. Although there was little evidence that viability of these marine-based industries has been affected by proximity of an urban-industrial center and surrounding intensive agricultural areas, it was felt that a survey providing information dealing with extent and distribution of persistent pesticides in the bay would provide data against which future investigations might be compared. It would also allow comparisons to be made between pesticide residue levels in this area and those in other regions.

Sampling

Sampling points were located as near as possible to mouths of streams flowing into the bay in the hope of determining the relationship between residue levels in the oysters and the land use of the catchment area (Fig. 1). All oysters were collected from rocks or mangrove roots in the intertidal zone. Table 1 describes individual sites and land use and population of the catchment area.

Analytical Procedures

Oysters were collected in their shells and kept on ice during transportation to the laboratory where they were stored at −4°C pending analysis.

Whole oyster-meat samples of approximately 10 g were submitted to the cleanup procedure for nonfatty foods described in the *Pesticide Analytical Manual* Vol. 1, Section 212.13a (1971) published by the Food and Drug Administration, U.S. Department of Health, Education, and Welfare. Appropriate adjustments were made for sample size. An aliquot of homogenized meat was extracted with acetonitrile and partitioned between hexane and the acetonitrile extract, which was diluted with water. The hexane phase was back-washed with water, dried, and cleaned in a florisil column packed with activated magnesium silicate.

A Varian 1400 gas chromatograph equipped with a tritium-foil electron-capture detector was used to identify and quantify pesticide residues on the sample extracts. Operating parameters were as follow:

Column:	DC-200 5 percent on Aeropak 30
Temperatures:	detector, 200°C; injector, 240°C; column, 185°C
Carrier gas:	nitrogen 40 ml/min

Recoveries of DDT, DDD, DDE, and dieldrin from samples fortified with 1 μg of each compound were 98, 93, 96, and 83 percent, respectively. Blank runs on solvents and reagents allowed a lower limit of reliable estimation to be set at 0.005 ppm. All data reported are corrected for recovery.

For confirmation of peak identity, the extract was concentrated to a low volume and a thin-layer chromatogram was run and compared with standards treated in a similar manner. At the levels under consideration, visualization of the spots was not possible and components were identified by scraping zones from the plate, extracting with hexane/ether, and injecting the extract into the gas chromatograph.

TABLE 1. *Australian sampling sites, land use, and population of catchment areas from which oysters were collected for monitoring, 1970-72*

STATION 1	
Site:	adjacent to small boat harbor. Samples from rock wall.
Industry:	dairy farming.
Population:	low-density rural, with small holiday settlement.
STATION 2	
Site:	rocky headland adjacent to estuarine area of bay. Samples from rock.
Industry:	sugar-cane farming, dairy farming, sugar mill, distillery.
Population:	predominantly rural; one town, Beenleigh.
STATION 3	
Site:	adjacent to small boat harbor. Samples from rocks.
Industry:	fruit and vegetable farming.
Population:	intensive rural; some outer-city suburbs.
STATION 4	
Site:	rocky headland near small boat harbor. Samples from rocks.
Industry:	principally suburban residential.
Population:	medium- to high-density urban.
STATION 5	
Site:	rock wall on river's edge near oil refinery. Samples from rocks.
Industry:	dairying and intensive agriculture in upper reaches of river. Industrial, commercial and high-density urban in lower reaches.
Population:	high density; major city, Brisbane.
STATION 6	
Site:	rocky headland adjacent to small boat harbor. Samples from rocks.
Industry:	residential and light industry.
Population:	high density.
STATION 7	
Site:	rocky headland. Samples from rocks.
Industry:	outer city residential, paper mill, pine forests, and some agriculture upstream.
Population:	medium density.
STATION 8	
Site:	mangrove trees in sandy silt. Samples from mangrove roots.
Industry:	dairying, pine forests.
Population:	medium- to low-density rural; one town, Caboolture, upstream.

Results and Discussion

Residue levels of DDT, DDD, DDE, and dieldrin calculated on a whole oyster-meat basis from eight samplings at each station are given in Table 2. DDT and dieldrin averages for the period of the survey are shown in graphic form in Figure 2.

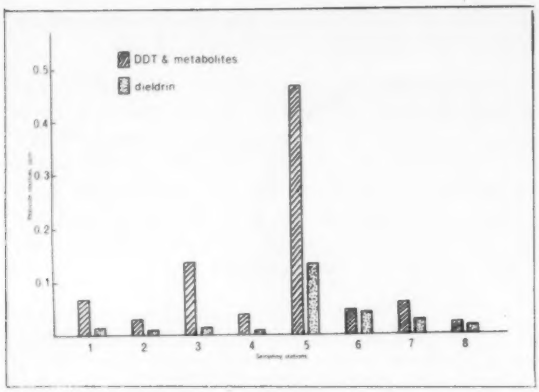

FIGURE 2. *Average pesticide residue levels at Australian oyster sampling stations 1-8, 1970-72*

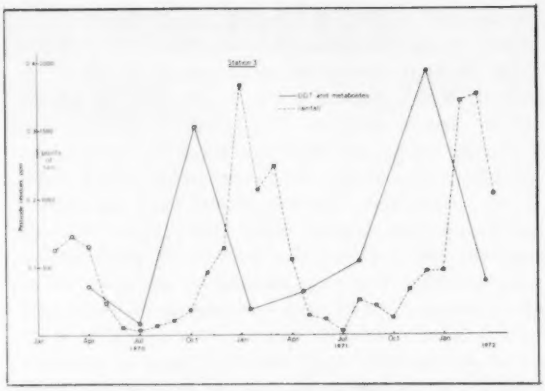

FIGURE 3. *Rainfall and total DDT levels at Australian oyster sampling station 3, 1970-72*

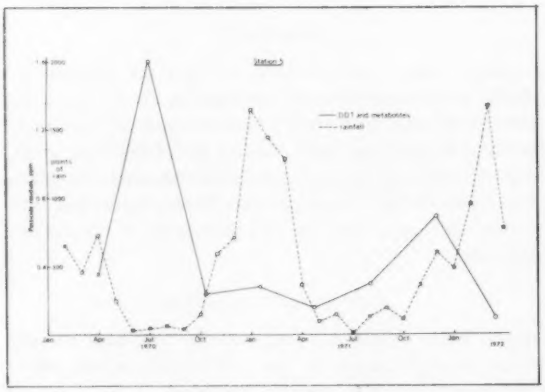

FIGURE 4. *Rainfall and total DDT levels at Australian oyster sampling station 5, 1970-72*

Highest individual residues, 0.94 ppm DDT, 0.51 ppm DDD, 0.20 ppm DDE, and 0.34 ppm dieldrin, were obtained in July 1970 at the Brisbane River Station, in whose catchment area lies the city of Brisbane, population 700,000. Second-highest levels were from station 3, which adjoins an area of land used intensively for vegetable and fruit production. Most other stations lie downstream from regions with considerably lower levels of human activity.

Stations 2 and 7 had levels which were somewhat unexpectedly low. The river adjoining station 2 passes through a cane-growing area where dieldrin is used to control root pests. Station 7 lies at the mouth of a stream on which a paper mill and pine forests are located.

Residue levels varied with the seasons. Two factors most likely to influence these variations are temperature and rainfall. Mean shade temperatures in this region range from about 26°C in December and January to about 16°C in June and July. A decrease in the pumping rate of the oyster in winter months would tend to lower both the rate of intake of material from the water and the rate of its elimination. Whether this would result in a net loss or gain in chlorinated hydrocarbons is not certain.

Reports commenting on seasonal variations do so mainly in terms of rainfall changes (*1-3*). In the region where this survey was conducted, the wet season occurs in the summer, December to March, although the winter is comparatively dry. Figures 3 and 4 show variation in rainfall and the fluctuation in total DDT group levels for stations 3 and 5. It is clear that residue maxima do not coincide with nor immediately follow periods of high rainfall. Other stations, while exhibiting irregularities in some cases, follow this general pattern.

This phase difference between rainfall maximum and residue level maximum may be explained in several ways. Rowe et al. interpret an increase in estuarine oysters near New Orleans as a result of an immediately preceding increase in runoff following heavy rains (*3*). In the present survey the rainfall maximum preceded the residue maximum by 5 to 11 months, generally with a dry period in between. Thus it is difficult to reconcile these data with the interpretation given below.

A general similarity in the pattern of residue variations found at most sampling stations suggests an alternative explanation. It is possible that the main source of residues in the region under investigation is the Brisbane River (station 5), and that distribution throughout the bay is effected by tidal diffusion.

However, this interpretation runs into at least two difficulties. The greatest similarity in the pattern of residue variations exists between station 5 and the streams to the south (stations 1-4); yet water from the Brisbane River flows predominantly northward. A second prob-

TABLE 2. *Chlorinated hydrocarbon levels in oysters* (Crassostrea commercialis), *Moreton Bay, Queensland, Australia*

Sampling Station	Sampling Date	Residues, ppm			
		DDE	DDD	DDT	Dieldrin
1	4-2-70	0.007	0.006	0.006	0.007
	7-2-70	—	—	—	—
	10-8-70	0.039	0.023	0.046	—
Coomera River	1-14-71	0.008	0.007	—	0.022
	4-22-71	0.014	0.007	0.038	0.047
	8-3-71	0.023	—	0.161	—
	11-29-71	0.011	0.007	0.068	0.005
	3-9-72	0.007	0.008	0.036	0.013
2	4-2-70	0.007	—	—	—
	7-2-70	0.055	—	—	—
	10-8-70	0.020	—	0.055	—
Logan River	1-14-71	0.006	0.007	—	0.027
	4-22-71	0.009	0.007	—	0.012
	8-3-71	0.014	—	0.022	—
	11-29-71	0.007	0.007	0.005	—
	3-9-72	—	—	—	0.010
3	4-2-70	0.022	0.020	0.029	0.009
	7-2-70	0.035	—	0.011	—
	10-8-70	0.035	0.010	0.26	—
Redland Bay	1-14-71	0.010	0.010	0.017	0.025
	4-22-71	0.016	0.014	0.034	0.032
	8-3-71	0.028	0.009	0.071	—
	11-29-71	0.046	0.025	0.318	0.006
	3-9-72	0.018	0.012	0.049	0.015
4	4-2-70	0.007	—	0.016	—
	7-2-70	—	—	—	—
	10-8-70	0.017	—	0.073	—
Tingalpa Creek	1-14-71	—	—	—	—
	4-22-71	0.010	0.007	0.019	0.022
	8-3-71	0.014	—	—	—
	11-29-71	0.018	0.013	0.061	—
	3-9-72	0.008	0.005	0.014	0.016
5	4-2-70	0.037	0.116	0.203	0.094
	7-2-70	0.20	0.51	0.94	0.34
	10-8-70	0.033	0.107	0.094	0.083
Brisbane River	1-14-71	0.072	0.057	0.150	0.069
	4-22-71	0.026	0.034	0.097	0.071
	8-3-71	0.045	0.085	0.168	0.116
	11-29-71	0.146	0.119	0.427	0.150
	3-9-72	0.026	0.019	0.054	0.111
6	4-2-70	0.009	—	0.013	0.020
	7-2-70	—	—	—	0.058
	10-8-70	0.020	0.014	—	0.057
Cabbage Tree Creek	1-14-71 [1]				
	4-22-71	0.039	0.022	0.022	0.058
	8-3-71	0.023	0.011	0.051	0.033
	11-29-71	0.028	—	0.046	0.027
	3-9-72	—	—	0.006	0.023
7	4-2-70	0.009	0.023	0.012	0.023
	7-2-70	0.016	—	—	—
	10-8-70	0.021	0.017	0.040	0.051
Pine Rivers	1-14-71	0.035	0.027	0.035	0.010
	4-22-71	0.021	0.009	0.014	0.032
	8-3-71	0.023	0.007	0.095	0.020
	11-29-71	0.005	—	0.030	0.014
	3-9-72	—	—	0.012	0.044
8	4-2-70	0.009	—	0.007	0.018
	7-2-70	0.059	—	—	—
	10-8-70 [1]				
Caboolture River	1-14-71	0.008	—	—	—
	4-22-71	0.009	—	—	0.013
	8-3-71	0.009	—	—	0.016
	11-29-71	0.006	—	0.006	0.012
	3-9-72	—	0.006	0.010	0.035

NOTE: — = < 0.005 ppm

[1] Large interfering peaks prevented analysis.

lem with this interpretation is that separation between the residue maximum and the rainfall maximum for each station would be expected to change to reflect increasing distance between a particular station and station 5. No such clear trend is observable.

A third hypothesis might also explain the data. If it is assumed that the amount of pesticide entering streams via sewerage outflows, industrial waste discharge, and irrigation systems does not vary greatly throughout the year, then the concentration in the water at the mouth

of the stream will be inversely related to the volume of fresh water flowing in that stream. Therefore one would expect the level of pesticide in the indicator organism to be highest during or shortly after the period when the flow is least, i.e., when the concentration of pesticide in the water is greatest.

Data for the DDT group totals at stations 1-5 fit this hypothesis satisfactorily; data for stations 6-8 moderately correlate. Dieldrin levels at station 5 also conform to this pattern.

Marked changes in the levels of DDT in oysters collected by Butler in 1966 in Tres Palacios Bay, Tex., were explained in this manner (*1*).

Ockham's razor notwithstanding, data for station 3, an area of intensive vegetable and tropical fruit cultivation, may also be interpreted in terms of the high seasonal rate of application of pesticides in the spring months of September and October.

Clearly, no single causative factor will adequately explain environmental data of this kind because the possibility of accidental spillages and intermittent high usage levels may and almost certainly do contribute to the distortion of otherwise regular patterns. Nevertheless it seems worthwhile to eliminate facile explanations and to try to attribute the data to some reasonable set of circumstances, thus providing a basis for comment and further investigation.

Overall levels obtained in this survey might usefully be compared with several published reports (*1-6*). In the major survey carried out by Butler's group in which data were collected over 5 years from 170 stations on the Atlantic, Gulf, and Pacific coasts of the United States, DDT and metabolites were usually less than 0.5 ppm even near intensively farmed areas, and only rarely greater than 1.0 ppm (*2,4*). The highest level found was 5.4 ppm; this was the result of a single incident of gross pollution. In other surveys, levels were generally of the same order of magnitude as those reported in this paper (*3,5,6*).

It would appear, then, that levels in this region were generally not atypical, although two samplings at station 5 could only be described as rare, in terms of the Butler survey.

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DDT and Dieldrin Residues in Selected Biota from San Antonio Bay, Texas—1972 [1]

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ABSTRACT

A total of 240 samples of 10 species of selected biota from San Antonio Bay, Tex., were collected and analyzed by electron-capture/gas-liquid chromatography for residues of the organochlorine insecticides DDT and its metabolites, and dieldrin. The most common residue detected was p,p'-DDE, which was the only member of the DDT group found in most samples. Results indicated that the DDT/dieldrin load in the biota sampled was relatively low; the greatest concentration was 106 ppb DDE in an oyster. Blue crabs had the greatest incidence of contamination, especially by DDE. Incidence of dieldrin contamination in blue crabs, oysters, and clams was similar. Of the major groups sampled, shrimp had the lowest incidence of DDT/dieldrin residues.

Introduction

The widespread occurrence of the organochlorine insecticides DDT and dieldrin as environmental pollutants has been well documented for all major terrestrial, freshwater, and marine environments. The presence of DDT and dieldrin in the water, sediments, and biota of Texas bays has been reported by Ahr (*1*), Butler (*2*), Childress (*3,4*), Fay and Newland (*5*), Flickinger and Meeker (*6*), Flickinger and King (*7*), and Petrocelli et al. (*8*). Although most of the former uses of DDT are now banned, there are still sufficient residues in the environment to warrant consideration of its effects (*9*). The significance of dieldrin as a pollutant in the environment has been well demonstrated: it has been the most common insecticide in all U.S. river basins since 1958 (*10,11*); it is more toxic than DDT to a number of organisms including salmonid fish (*12,13*), American oyster larvae (*14*), and wildlife (*15*); and it is extremely persistent in the environment (*16*). Dieldrin, an insecticide in its own right, also results from the oxidative breakdown (epoxidation) of aldrin, a closely related insecticide (*17*). Both DDT and dieldrin are accumulated by aquatic organisms (*18-21*) and biologically magnified in food chains (*22-27*).

The San Antonio Bay system is located just south of the geographic center of the Texas Gulf Coast (Fig. 1) between 28°00′ and 28°30′ N latitude and 96°30′ and 97°00′ W longitude in Calhoun and Refugio Counties. The Aransas National Wildlife Refuge is on the west side of the bay (Fig. 2). San Antonio Bay is a commercial fisheries area with significant landings of penaeid shrimp, American oysters, blue crabs, and a wide variety of fin fish.

Sample Collecting

March clams (*Rangia cuneata*) were collected at a depth of 2-3 feet along the shoreline just above Grassy Point in Hynes Bay; eastern oysters (*Crassostrea virginica*) were from Panther Point reef; blue crabs (*Callinectes sapidus*) were collected in traps in the mouth of Hynes Bay off McDowell Point and from trawls taken in various parts of San Antonio Bay; brown shrimp (*Penaeus aztecus*) were also taken from trawls. Miscellaneous samples, which included two species of fish: Bay anchovies (*Anchoa mitchilli*) and southern flounders (*Paralichthys lethostigma*); bivalved molluscs (*Mercenaria campechiensis, Tagelus plebeius,* and *Macoma constricta*); and marsh grass (*Spartina spartinae*), were from areas along the Aransas National Wildlife Refuge shore and islands along the Intracoastal Waterway. All samples were placed on ice in appropriate containers immediately after collection until returned to the laboratory where they were analyzed or frozen at −15° C until analysis.

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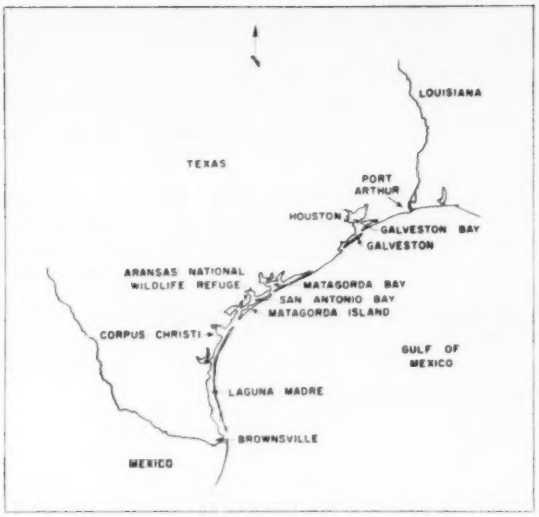

FIGURE 1. *Texas coast, Gulf of Mexico, with San Antonio Bay system indicated*

Analytical Procedures

Samples were analyzed for DDT and dieldrin residues by a modification of the procedures of the U.S. Department of Health, Education, and Welfare, Food and Drug Administration, as described in the *Pesticide Analytical Manual* (*28*). Insecticide residues were extracted from tissues by blending with Mallinckrodt nanograde acetonitrile for 5 minutes at high speed. The resulting mixture was filtered through sharkskin filter paper. The filter paper and tissue residue were re-extracted with acetonitrile and filtered as before. Filtrates were combined and 200 ml were partitioned in 100 ml nanograde petroleum ether in a separatory funnel. The aqueous layer was discarded and the solvent layer washed twice with a 2 percent sodium sulfate solution and dried by the addition of anhydrous sodium sulfate.

The dried extract was concentrated to a volume of 10 ml on a steam bath and placed on a florisil column for cleanup. The amount of florisil used was determined by lauric acid titration (*28*). Residues were eluted individually from the florisil column by 6, 15, and 50 percent solutions of diethyl and petroleum ether. Residue analyses were performed on a Barber-Colman Selecta-System Series 5000 gas-liquid chromatograph (GLC) fitted with an electron-capture detector. The column was a 2m-by-4mm-ID Pyrex glass coil, packed with 80/100 mesh Gas Chrom Q support on which the liquid phase of 3 percent OV-1 was distributed. The carrier gas was prepurified nitrogen and the operating temperatures were: injector, 200° C; column, 180° C; and detector, 210° C. Minimum detectable level for these organochlorine compounds was 0.001 ppm.

Residue peaks were qualified by retention time relative to a standard, and quantification was performed by peak height measurements. Confirmations of random sample residues were performed on a second GLC column (DC-200) and by p-values (*28*). Multiple peaks characteristic of polychlorinated biphenyls (PCB's) were not detected in any samples. Residue concentrations are expressed as parts per billion (ppb, μg/kg) on a wet-weight, whole-animal basis. Recoveries of pesticide standards obtained from Shell Chemical Company carried through the analytical procedure were consistently over 85 percent and often over 90 percent. No corrections for percent recovery have been made in reporting these data.

Results and Discussion

Results of analyses indicated a relatively low DDT/dieldrin load in biota of San Antonio Bay (Tables 1-3). Of 240 samples analyzed, 159 (66 percent) contained residues of ΣDDT, which were almost exclusively *p,p'*-DDE; 70 (29 percent) contained dieldrin residues, and 47 (20 percent) contained residues of ΣDDT and dieldrin (Table 3). The greatest incidence of ΣDDT and dieldrin was in blue crabs: 94 percent contained residues of ΣDDT and 32 percent contained residues of dieldrin. Oysters and clams, the two major bivalved mollusc groups sampled, had a similar incidence of dieldrin residues: 25 percent of the oysters and 31 percent of the clams had detectable residues. Compared with blue crabs, however, the bivalves' incidence of total DDT was much lower: 60 percent of the oysters and 68 percent of the clams contained these residues. In 1973 Butler reported detecting residues of DDE without residues of DDT or TDE in oysters collected the preceding year from San Antonio Bay (*2*); the present study revealed similar findings in oysters. This predominance of DDE residues in tissues may reflect decreasing DDT input over the years; conversion of DDT to its common metabolite, DDE, which occurs in many aquatic species (*17*); and the biocycling of these residues. Shrimp had a comparatively low incidence of DDT/dieldrin residues.

High incidence of ΣDDT in blue crabs may be caused by their omnivorous or opportunistic feeding habits. Blue crabs, which scavenge on dead fish, also eat a wide variety of living organisms: fish; benthic invertebrates such as *Rangia* clams, oysters, and snails; and vascular plants and organic detritus (*29*). Therefore high incidence of ΣDDT in blue crabs may result from their ingestion of a wide variety of insecticide-contaminated materials. Bivalves, on the other hand, are more selective, feeding on suspended detritus, protozoans, and plankton for at least 70 percent of their

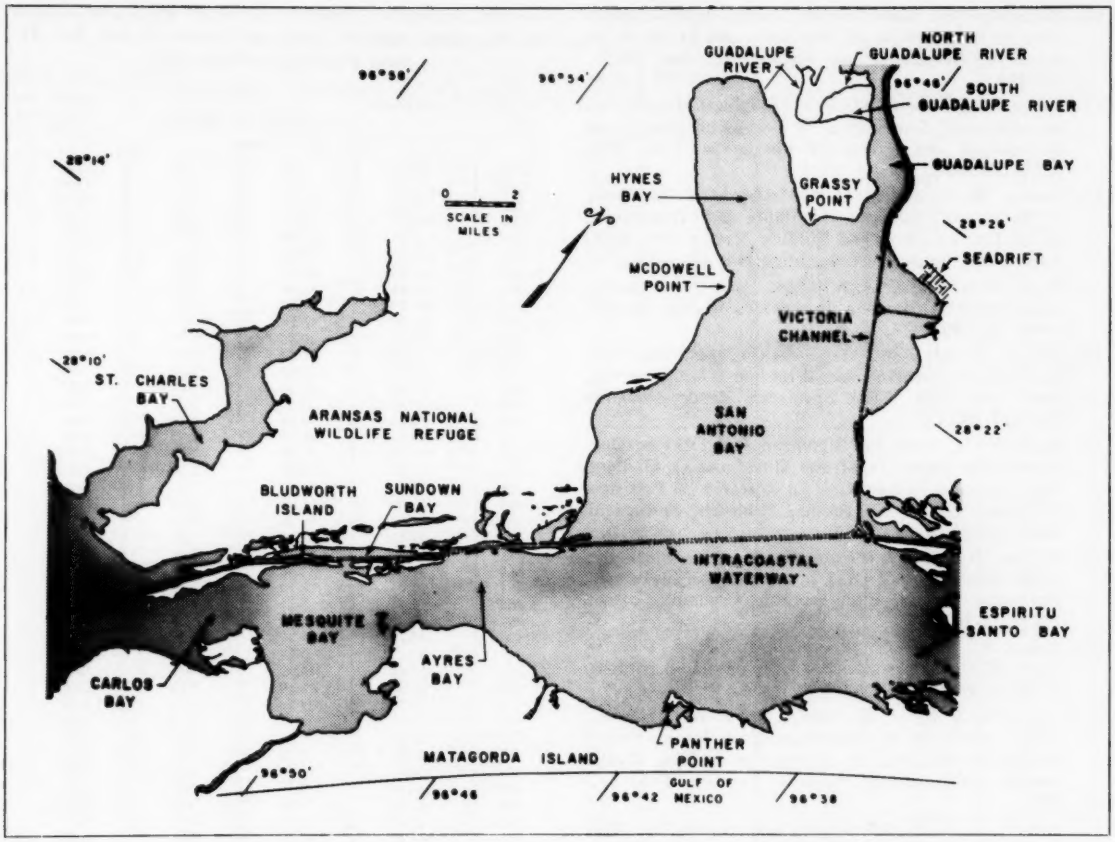

FIGURE 2. *San Antonio Bay system and Aransas National Wildlife Refuge*

nourishment (*29*). Hence they have less overall exposure to insecticide residues than have blue crabs, in spite of the bivalves' well-known ability to concentrate insecticides from water into their tissues. Furthermore, because blue crabs may undertake migrations, they have many more opportunities to be exposed than have bivalves.

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TABLE 1. *Baseline residues (μg/kg) of DDT and dieldrin in blue crabs, oysters, clams, and brown shrimp, San Antonio Bay System, Tex.—1972*

Sample No.[1]	Wet Weight, g	*p,p'*-DDE	Dieldrin	Month of Collection
Blue Crabs				
219	98.24	9.47	1.44	April
220	144.86	9.73	ND	April
225[2]	77.28	28.32	4.22	April
226	147.53	22.13	1.38	April
227	113.41	11.14	1.97	April
228	103.91	29.50	6.16	April
229	161.85	20.20	3.27	April
230	94.64	19.40	3.56	April
231	193.84	18.54	3.22	April
303	159.96	13.80	2.76	June
304	137.81	10.80	TR	June
306	48.75	22.80	ND	June
307	66.54	17.60	2.12	June
308	17.39	40.30	ND	June
309	75.32	12.20	TR	June
310	79.63	9.95	ND	June
311	29.51	25.80	3.47	June
312	11.30	28.00	ND	June
313	68.85	19.00	2.96	June
314	54.50	13.10	TR	June
330	91.39	16.30	20.70	June
331	31.53	16.30	ND	June
332	29.62	51.20	5.41	June
333	62.22	13.70	2.69	June
366	41.24	23.90	ND	July
367	27.03	18.10	2.75	July
368	5.95	ND	ND	July
369	9.68	ND	ND	July
370	40.37	19.30	3.50	July
371	23.82	ND	5.76	July
372	10.68	14.90	ND	July
373	11.93	13.10	ND	July
374	22.26	27.70	44.60	July
375	37.61	ND	2.64	July
376	29.68	ND	4.41	July
383	146.91	11.70	1.30	July
384	100.62	11.40	1.01	July
385	126.48	8.77	ND	July
386	117.72	9.66	ND	July
387	65.13	16.30	ND	July
395	148.89	68.20	ND	July
396[3]	296.38	29.80	ND	July
397	116.94	3.13	ND	July
398	44.39	21.60	ND	July
410	396.90	4.43	ND	July
411	168.17	12.10	ND	July
412	196.48	10.00	ND	July
413	163.05	6.54	ND	July
518	87.69	13.60	ND	October
519	74.28	6.20	ND	October
520	59.01	13.40	ND	October
521	77.06	5.90	ND	October
522	93.74	18.70	ND	October
523	75.53	15.80	ND	October
584[4]	64.95	15.00	ND	October
585[5]	53.88	6.20	ND	October
586[6]	56.74	6.30	ND	October
587[7]	58.58	55.00	ND	October
588[4]	93.78	5.20	ND	October
589[7]	62.77	4.30	ND	October
590[6]	51.85	15.00	ND	October
591[7]	63.76	8.10	ND	October
Oysters				
213	10.85	29.85	9.28	April
214	5.17	46.59	19.62	April
215	4.16	28.18	22.91	April
216	9.31	27.11	13.38	April
234	7.70	51.87	27.11	April
235	14.10	29.76	13.14	April
236	2.90	72.25	31.90	April
237	2.12	70.17	36.08	April
294[8]	28.81	ND	ND	June
295[9]	131.73	3.74	ND	June
405	3.48	28.50	ND	June
406	2.73	28.90	ND	June
407	7.14	28.80	ND	June
408	9.15	28.40	ND	June
409	3.96	37.30	ND	June
551	8.39	27.00	ND	July

(Continued next page)

TABLE 1 (cont'd). *Baseline residues (μg/kg) of DDT and dieldrin in blue crabs, oysters, clams, and brown shrimp, San Antonio Bay System, Tex.—1972*

Sample No.[1]	Wet Weight, g	*p,p'*-DDE	Dieldrin	Month of Collection
Oysters, cont'd				
552	7.01	ND	22.20	July
553	7.38	106.00	20.60	July
554	8.57	24.40	24.20	July
555	7.73	ND	ND	July
556	5.56	ND	ND	July
557	7.08	ND	ND	July
558	2.55	ND	ND	July
559	3.26	ND	ND	July
560	4.14	ND	ND	July
592	2.42	ND	26.00	October
593	3.21	23.00	ND	October
594	5.51	25.00	ND	October
595	5.60	48.00	ND	October
596	7.53	ND	ND	October
Clams				
221	10.89	18.42	13.73	April
222	13.64	22.86	ND	April
296[10]	33.81	21.90	ND	June
297[11]	56.27	18.40	ND	June
339	21.51	25.40	ND	June
340	23.18	37.00	6.65	June
341	16.42	36.70	ND	June
342	18.68	32.90	3.73	June
343	18.25	42.40	3.75	June
344	20.62	38.70	3.16	June
346	11.75	42.80	9.34	June
347	9.06	59.90	1.07	June
348	10.69	50.20	12.40	June
349	6.28	ND	13.90	June
356	10.85	34.70	3.53	June
357	5.36	ND	ND	June
358	11.63	54.80	2.50	June
359	10.96	35.80	ND	June
360	10.18	ND	ND	June
541[12]	3.20	ND	ND	September
542	3.91	ND	ND	September
543	4.63	ND	ND	September
544	7.80	ND	ND	September
561	6.50	36.00	14.00	April
562	7.55	30.00	12.00	April
563	6.36	ND	ND	April
Clams, cont'd				
564	6.22	43.00	ND	April
565[13]	7.83	15.00	ND	April
566	7.52	ND	ND	April
567	6.99	10.00	ND	April
568	9.20	ND	ND	April
569	7.83	ND	ND	April
570	7.02	ND	5.10	April
571[14]	18.67	ND	2.00	April
598	6.30	ND	ND	October
599	4.71	18.00	ND	October
600	4.58	ND	18.00	October
601	3.65	ND	ND	October
602	2.82	79.00	ND	October
603	9.74	20.00	ND	October
605	8.12	ND	ND	October
606[15]	5.92	16.00	ND	October
607	12.33	16.00	ND	October
Brown Shrimp				
240	6.48	ND	6.70	April
241	7.82	ND	4.51	April
242	8.16	ND	4.38	April
249	6.71	ND	8.71	April
250	9.44	ND	ND	April
251	5.64	ND	6.50	April
252	10.15	ND	ND	April
254	5.73	ND	11.36	April
256	8.87	ND	ND	April
257	6.59	ND	ND	April
258	12.40	ND	ND	April
259	6.74	ND	ND	April
266	4.23	ND	ND	May
267	3.80	ND	ND	May
268	6.20	ND	ND	May
269	4.81	ND	ND	May
270	3.53	ND	ND	May
271	5.98	ND	ND	May
272	5.08	ND	ND	May
273	10.65	ND	ND	May
402	5.60	15.70	ND	July
403	5.52	14.70	ND	July
404	5.58	13.70	ND	July

NOTE: Residue totals and averages listed in Table 3.
ND = not detected.
TR = trace (< 1.0 μg/kg).

[1] Samples represent individual specimens unless indicated otherwise.
[2] Sample taken from Bludworth Island.
[3] Sample contained 18.60 μg/kg *p,p'*-DDE.
[4] Composite of two crabs.
[5] Composite of six crabs.
[6] Composite of four crabs.
[7] Composite of three crabs.
[8] Composite of ten small oysters.
[9] Composite of ten large oysters.
[10] Composite of ten small clams.
[11] Composite of ten large clams.
[12] Sample taken from Sundown Bay.
[13] Sample contained 33.00 μg/kg *p,p'*-DDE.
[14] Composite of five clams.
[15] Sample contained 47.00 μg/kg *p,p'*-DDE.

TABLE 2. *Baseline residues (μg/kg) of DDT and dieldrin in miscellaneous species, San Antonio Bay System, Tex.—1972*

Sample No.	Organism [1]	Location [2]	Wet Weight, g	*p,p'*-DDE	Dieldrin	Month of Collection
223	*Anchoa mitchilli*	Bludworth Island	32.80	52.61	5.38	April
224	*Tagelus plebeius*	Bludworth Island	29.84	5.67	2.68	April
238	*Paralichthys lethostigma*	Refuge	14.72	13.32	ND	April
239	*Paralichthys lethostigma*	Refuge	14.78	14.21	ND	April
435	*Mercenaria campechiensis*	Hynes Bay	75.95	ND	ND	July
436	*Mercenaria campechiensis*	Hynes Bay	34.28	ND	ND	July
437	*Mercenaria campechiensis*	Hynes Bay	55.66	ND	ND	July
438	*Mercenaria campechiensis*	Hynes Bay	25.41	ND	ND	July
439	*Mercenaria campechiensis*	Hynes Bay	42.58	ND	ND	July
450	*Tagelus plebeius*	Refuge	4.75	ND	ND	July
451	*Tagelus plebeius*	Refuge	8.07	ND	ND	July
452	*Macoma constricta*	Refuge	2.83	ND	ND	July
526	*Spartina spartinae*	Dunham Bay	57.43	2.20	ND	August
527	*Spartina spartinae*	Mustang Lake	57.25	ND	ND	August
528	*Spartina spartinae*	Dunham Bay	49.11	ND	ND	August
533	*Mercenaria campechiensis*	Hynes Bay	95.26	ND	1.30	October
534	*Mercenaria campechiensis*	Hynes Bay	135.69	ND	1.20	October
535	*Mercenaria campechiensis*	Hynes Bay	109.45	ND	1.40	October
536	*Mercenaria campechiensis*	Hynes Bay	155.47	ND	1.00	October
537	*Mercenaria campechiensis*	Hynes Bay	20.43	ND	ND	October
538	*Mercenaria campechiensis*	Hynes Bay	11.52	ND	ND	October
539	*Mercenaria campechiensis*	Hynes Bay	17.95	ND	ND	October
540	*Tagelus plebeius*	Sundown Bay	4.24	ND	ND	September

[1] See text for common names.
[2] Compare map, Fig. 2.

TABLE 3. *Summary of insecticide residues detected in biological samples, San Antonio Bay System, Tex.—1972*

Species	No. Organisms Analyzed	No. Organisms Containing Insecticide Residues and Percent Total Analyzed		
		p,p'-DDE	Dieldrin	Both
Blue crabs	81	76 (94%)	26 (32%)	23 (28%)
Oysters	48	29 (60%)	12 (25%)	10 (21%)
Clams	65	44 (68%)	20 (31%)	12 (18%)
Shrimp	23	3 (13%)	6 (26%)	0 (0%)
Miscellaneous	23	7 (30%)	6 (26%)	2 (9%)
Total	240	159 (66%)	70 (29%)	47 (20%)

Residues of 2,4-D in Pond Waters, Mud, and Fish, 1971 [1,2]

Donald P. Schultz and Paul D. Harman

ABSTRACT

Nine ponds in Florida, Georgia, and Missouri were treated in 1971 with the dimethylamine salt of 2,4-Dichlorophenoxyacetic acid (DMA-2,4-D) to determine residue levels of the herbicide in water, bottom mud, and fish as a function of application rate, time, substrate, and geographic location. The acid equivalent of 2,4-D was applied at treatment rates of 2.24, 4.48, or 8.96 kg/ha. Samples were taken up to 147 days after treatment.

Residues of 2,4-D in pond waters declined from a maximum of 0.345 and 0.692 mg/liter in Florida and Georgia, respectively, to less than 0.005 mg/liter 28 days after treatment, and from 0.630 mg/liter to less than 0.005 mg/liter 56 days after treatment in Missouri pond waters.

Residues in mud from the Florida and Georgia ponds never exceeded 0.05 mg/kg and had declined to trace or nondetectable levels 56 days after treatment. The highest residue found was 0.170 mg/kg from the first- and third-day samples of mud in the most heavily treated Missouri pond. In mud from one Missouri pond, residues were detected as late as 28 days after treatment; no residues occurred in any ponds after that.

No evidence of fish kill was found in any ponds treated with DMA-2,4-D. Of 307 fish samples analyzed, 45 contained detectable residues of 2,4-D. Residue levels ranged from slightly more than 1.0 mg/kg to less than 0.010 mg/kg.

Introduction

Proliferation of obnoxious aquatic plants is presently controlled by mechanical, physical, biological, or chemical means, or by combinations of these methods. Formulations of 2,4-D are used most widely to control plants such as the waterhyacinth (*Eichornia crassipes*) and eurasian watermilfoil (*Myriophyllum spicatum*). In 1966 the Tennessee Valley Authority applied 888 tons of the butoxyethanol ester of 2,4-D to 8,000 acres of watermilfoil in several reservoirs (*1*). Over 18,000 surface acres of Nickajack and Guntersville Reservoirs on the Tennessee River were treated with about 170,000 gallons of the dimethylamine salt of 2,4-D (DMA-2,4-D) April—June 1969, to control watermilfoil (*2*).

Objectives of the present study conducted in 1971 were to determine the residue levels and rate of dissipation of DMA-2,4-D in fish, water, and bottom mud. These data are required to register the product for use in aquatic plant control and to assist in establishing residue tolerances for DMA-2,4-D in fish and domestic water. Also, by using ponds in Florida, Georgia, and Missouri, authors hoped to determine whether different physical and chemical characteristics of the aquatic environment affect the uptake and dissipation of the herbicide. A third objective was to assess the efficacy of the herbicide on minor infestations of waterhyacinth in Florida and Georgia ponds.

Methods and Materials

SITE SELECTION

Three ponds were located near Crystal River, Fla. Willow Pond contained some aquatic plants including *Hydrocotyle* sp., cattail (*Typha* sp.), waterlettuce (*Pistia stratiotes*), widgeongrass (*Ruppia maritima*), and *Sagittaria* sp. in about 5 percent of its total area. One bank was heavily covered with willow (*Salix* sp.). Shelter Pond contained a dense stand of *Chara* sp., which covered about one-third of the bottom, and *Hydrilla verticillata*, which covered about 50 percent of the pond area at the beginning of the experiment. The third pond, designated 11-T, contained small amounts of widgeongrass, *Sagittaria* sp., and *Hydrocotyle* sp. Initially, about 30 percent of its area contained

[1] Southeastern Fish Control Laboratory, Fish and Wildlife Service, U.S. Department of the Interior, Warm Springs, Ga. 31830.
[2] Supported in part by the U.S. Army Corps of Engineers.

Hydrilla sp. All three ponds were treated with rotenone in May 1971 and restocked in June 1971 with largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*), and redear sunfish (*Lepomis microlophus*). One month prior to treatment the three ponds were stocked with waterhyacinth so that it would cover 5-10 percent of surface area of each pond at the time of treatment.

A second study area of four ponds was located within a 10-mile radius of Warm Springs, Ga., on the Piedmont Plateau and was comprised of four ponds which contained little, if any, submersed vegetation. Several of the ponds had small stands (less than 5 percent coverage) of cattail. Because the ponds contained established fish populations of the desired species, only enough fish were added to ensure an adequate number for the experiment. Waterhyacinths were transported from Florida to stock the ponds; their total coverage did not exceed 5 percent of the surface area of each pond. The ponds were designated 0, 2, 4, and 8 in accordance with the amount of herbicide applied.

Four ponds representing a third ecological and geographical type were located at the USDI Fish and Wildlife Service, Fish-Pesticide Research Laboratory, Columbia, Mo. Pond 9 contained no emersed or submersed macrophytes because of its prior use as a crayfish holding pond. Pond 15, the control, contained a common grass (*Avena* sp.) which had established itself when the pond was dry. About 5 percent of the surface area of Pond 28 contained cattails. There was also a 20-30 percent infestation of an unidentified filamentous alga. About 10 percent of the surface area of Pond 26 contained smartweed (*Polygonum* sp.). Physical characteristics of the ponds used in the experiment are listed in Table 1.

TABLE 1. *Physical characteristics of ponds in Florida, Georgia, and Missouri, and initial 2,4-D concentrations, 1971*

Pond	Surface area, acres	Volume, acre-ft	Depth, ft		2,4-D, mg/L
			Average	Maximum	
Fla-Willow	0.60	2.54	4.23	7.0	0.174
Fla-Shelter	0.43	1.45	3.37	6.0	0.436
Fla-11-T	0.80	3.12	3.90	8.0	0.801
Ga-0	1.30	7.30	5.60	11.0	0.000
Ga-2	0.60	2.65	4.40	8.0	0.166
Ga-4	0.90	2.70	3.00	7.0	0.490
Ga-8	0.86	2.95	3.40	7.0	0.857
Mo-15	0.073	0.166	2.26	4.5	0.000
Mo-9	0.073	0.166	2.26	4.5	0.444
Mo-28	0.162	0.368	2.26	4.0	1.002
Mo-26	0.140	0.451	3.20	4.5	1.631

NOTE: Florida ponds had sandy bottoms covered with a muck layer 1-3 inches deep, Georgia ponds had red clay bottoms characteristic of the Piedmont Plateau, and Missouri ponds had bottoms of heavy colloidal clay.

TREATMENT

DMA-2,4-D was applied to nine ponds at 2.24, 4.48, or 8.96 kg per hectare (kg/ha.) (2, 4, or 8 lb/acre) using a commercial formulation of herbicide (Weedar 64, Amchem Products, Ambler, Pa.) which contained 4 lb 2,4-D acid equivalent (a.e.) per gallon. All dilutions were made with water and no adjuvants were used. The Florida ponds were sprayed July 12, 1971, and the Georgia and Missouri ponds were sprayed July 26, 1971.

SAMPLING

Samples of fish, water, and mud were taken at 0, 1, 3, 7, 14, 28, 56, 84 or 86, 112, and 140 or 147 days after treatment. Fish were placed in live-cages in the Florida ponds for first- and third-day samples. Thereafter, fish were collected by hook and line, seine, or setline; they were weighed, measured, wrapped in aluminum foil, bagged, and frozen on dry ice. Water samples were taken with a 2-liter Kemmerer water bottle; they were composites of samples from areas whose depths varied from shallow (1 foot) to medium (2-3 feet) to deep (4 feet or more). The pH of water samples was determined either colorimetrically or with a pH meter. Water for residue analysis was placed in quart jars and acidified to a pH of less than 2 with concentrated sulfuric acid. Jars were capped with aluminum foil and sealed with screw caps.

Mud samples were taken with an Ekman dredge from shallow, medium, and deep sites. The three samples were composited for residue analysis, placed in plastic bags, and frozen on dry ice until analysis.

Analytical Procedures

Acidified water samples were extracted with chloroform which was subsequently evaporated. The 2,4-D residues were derivatized with diazomethane (*3*) for quantification by gas-liquid chromatography (GLC). Mud samples were acidified and extracted in a blender with a mixture of acetone, petroleum ether, and diethyl ether. The slurry was filtered and extracted twice with base (*4*). The basic solution was acidified and extracted with chloroform and the chloroform solution was treated as above. Fish samples were ground with dry ice (*5*) and the resultant powder was packed in a column and extracted with acidified methanol (*6*). The methanol eluate was added to water, the pH was adjusted to 1.5, and the solution was extracted with diethyl ether. The diethyl ether extract was treated as described earlier for chloroform extracts. Esterified 2,4-D residues were cleaned up on a column of silica gel. The gas chromatograph used to quantify 2,4-D residues was equipped with an electrolytic conductivity detector (*7*). Recovery values from spiked water, mud, and fish samples were 97.5 ± 2.5, 90 ± 2.5, and 90 ± 2.5, respectively.

All solvents used for extractions were glass-distilled (Burdick and Jackson, Muskegon, Mich.). All other chemicals used were analytical reagent grade. Residue levels are given in terms of the methyl ester of 2,4-D and were not corrected for percent recovery.

The detection limit of the 2,4-D methyl ester was 1.25 ng; 15 ng registered a 50 percent deflection on the recorder. Detection limits were 0.01 mg/kg, 0.005 mg/kg, and 0.001 mg/liter for fish, mud, and water samples, respectively.

Confirmatory analyses of the 2,4-D methyl ester standard, spiked samples, and periodic samples were conducted by electron-capture detection on a Beckman GC-4 gas chromatograph.

Detailed procedures for extraction of 2,4-D residues from water, mud, and fish samples, as well as the derivatization and gas chromatographic procedures, have been described by Schultz and Whitney (*7*).

Results

WATER

In five of the nine treated ponds, highest residue levels were detected the third day after treatment. Presumably it took this length of time for the herbicide to be thoroughly dispersed in the water. The highest level of 2,4-D residues in Florida pond water was 0.345 mg/liter; it was found 3 days after herbicide application to Pond 11-T, which was treated at 8.96 kg/ha. (8 lb/acre). Residue levels of 2,4-D in Florida ponds decreased to no more than 0.005 mg/liter within 14 days after treatment. Thereafter, only trace amounts (less than 0.005 mg/liter) or no residues were detected (Table 2).

In Georgia pond water the highest detectable residue was 0.692 mg/liter found in Pond 8 three days after treatment at 8.96 kg/ha. Residue levels of 2,4-D in these waters decreased to trace amounts (less than 0.005 mg/liter) within 28 days after treatment; no residues were detected thereafter (Table 2).

TABLE 2. *Residues of DMA-2,4-D in water (mg/L) and mud (mg/kg) samples from ponds treated with 2,4-D, 1971*

Treatment, pond, and sample	Days after treatment									
	0	1	3	7	14	28	56	84/86 [1]	112	140/147 [2]
FLORIDA										
2.24 kg/ha. (Willow Pond)										
water	ND	0.125	0.025	TR	TR	TR	TR	TR	ND	ND
mud	ND	TR	TR	0.005	TR	ND	ND	ND	ND	ND
4.48 kg/ha. (Shelter Pond)										
water	ND	0.155	0.172	0.048	0.005	TR	ND	TR	ND	ND
mud	ND	0.014	0.014	0.010	0.010	0.007	ND	TR	ND	ND
8.96 kg/ha. (11-T)										
water	ND	0.312	0.345	0.025	0.005	TR	ND	TR	ND	ND
mud	ND	0.033	0.046	0.008	0.013	ND	TR	TR	TR	ND
GEORGIA										
No herbicide (Pond 0)										
water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
mud	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2.24 kg/ha. (Pond 2)										
water	ND	0.025	0.087	0.059	0.027	TR	ND	ND	ND	ND
mud	ND	0.018	0.008	0.010	0.006	TR	ND	ND	ND	ND
4.48 kg/ha. (Pond 4)										
water	ND	0.233	0.390	0.400	0.050	TR	ND	ND	ND	ND
mud	ND	0.024	0.018	[3]	TR	ND	ND	ND	TR	ND
8.96 kg/ha. (Pond 8)										
water	ND	0.617	0.692	0.395	0.008	TR	ND	ND	ND	ND
mud	ND	0.026	0.040	0.042	TR	ND	ND	ND	ND	ND
MISSOURI										
No herbicide (Pond 15)										
water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
mud	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2.24 kg/ha. (Pond 9)										
water	ND	0.104	0.108	0.102	0.050	0.017	ND	ND	ND	ND
mud	ND	ND	TR	0.009	ND	ND	ND	ND	ND	ND
4.48 kg/ha. (Pond 28)										
water	ND	0.160	0.256	0.250	0.480	0.150	ND	ND	ND	ND
mud	[3]	0.011	0.012	0.005	TR	ND	ND	ND	ND	ND
8.96 kg/ha. (Pond 26)										
water	ND	0.580	0.420	0.326	0.630	0.135	ND	ND	ND	ND
mud	[3]	0.170	0.170	0.091	0.068	0.005	ND	ND	ND	ND

NOTE: ND = not detectable; TR = trace (less than 0.005 mg/L or kg).

[1] In Florida and Georgia the seventh posttreatment sampling was made 84 days after application; in Missouri it was made 86 days after application.

[2] In Florida and Georgia the last sampling was made 140 days after application; in Missouri it was made 147 days after application.

[3] Sample was lost.

The most persistent residues were found in the two most heavily treated Missouri ponds which contained more than 0.1 mg/liter of 2,4-D 28 days after treatment (Table 2). However, no herbicide was detected 56 days after treatment or thereafter.

MUD

Highest 2,4-D residues in mud were consistently found in the ponds which had received the heaviest herbicide treatment. The largest 2,4-D residue detected in mud from Florida ponds was 0.046 mg/kg found in Pond 11-T three days after treatment (Table 2). Trace amounts were found in the same pond up to 112 days after treatment. In the Georgia ponds the highest residue found was 0.042 mg/kg in Pond 8 seven days after treatment (Table 2). A residue level of 0.170 mg/kg from Missouri Pond 26 was found at the first- and third-day samplings; no residues were detected in any other Missouri pond 56 days after treatment or thereafter (Table 2).

FISH

Highest residues found in fish were from the first-day harvest of Willow and Shelter Ponds in Florida (Table 3). These fish were kept in live-cages during and after treatment to facilitate sampling; hence they were unable to escape from the applied herbicide (*8*). No residues were detected in fish from Florida ponds during the third- or seventh-day harvests; however, residues were detected 14 days after treatment. This may have been caused by release of the herbicide from decaying vegetation. Only one fish from the Florida ponds contained a detectable residue after 14 days. It was one of two largemouth bass from Pond 11-T, which had been treated at 8.96 kg/ha.; the residue was a trace amount (0.005 mg/kg).

The highest 2,4-D residue found in fish from the Georgia ponds was 0.075 mg/kg in one of three bluegills harvested 14 days after pesticide application at 8.96 kg/ha. (Table 3). No detectable residues were found in any fish from the Georgia ponds at the third- or seventh-day harvests; this parallels results of Florida pond samples. With one exception, the last detectable residues were found 28 days after treatment. The exception was a bluegill from Pond 8: it contained less than 0.005 mg/kg 84 days after treatment.

No residues of 2,4-D were detected in fish from the Missouri ponds until 28 days after treatment (Table 3). At that time all five bluegills sampled and one of five largemouth bass sampled had less than 0.005 mg/kg. No other fish from the Missouri ponds contained detectable residues of 2,4-D.

None of the fish from control ponds contained detectable residues of 2,4-D. The four highest residues were in fish from the first-day harvest which had been held in live-cages in the Florida ponds. The 2,4-D residues found in the exposed fish were well below the levels of the dimethylamine salt formulation found to be toxic to fish (*9-11*).

WATERHYACINTH CONTROL

The effect of the 2,4-D application on waterhyacinth in the Florida and Georgia ponds was assessed by visual observation. Nearly all the waterhyacinth was brown and decomposing 7 days after spraying. Approximately 98 percent of the plants had been killed by the herbicide application; no difference in kill was noted among the different treatment levels. Spot retreatment was needed in some areas of all ponds to prevent reinfestation from the estimated 2 percent of the waterhyacinth which survived the initial application.

TABLE 3. *Residues of DMA-2,4-D in fish from ponds treated with 2,4-D, 1971*

Species and fish number	Days after treatment	Residues (mg/kg) in fish treated at—			
		0 kg/ha.	2.24 kg/ha.	4.48 kg/ha.	8.96 kg/ha.
FLORIDA					
LMB-1	0	ND			
LMB-2	0	ND			
CCF	0	ND			
LMB-1	1		0.080	0.048	TR
LMB-2	1				ND
LMB-3	1				0.008
CCF-1	1		1.075	0.340	ND
CCF-2	1				ND
CCF-3	1				ND
BLG-1	1		0.024	0.420	0.010
BLG-2	1				TR
BLG-3	1				0.012
LMB	3		ND	ND	ND
CCF	3		ND	ND	ND
BLG	3		ND	TR	ND
LMB	7	ND	ND	ND	ND
CCF	7	ND	ND		ND
BLG	7	ND	ND	ND	ND
LMB-1	14	ND	0.036	ND	0.043
LMB-2	14		0.031		ND
LMB-3	14		TR		
CCF-1	14		0.029	0.050	0.024
CCF-2	14		0.032	0.012	0.102
CCF-3	14		0.012	0.039	0.094
BLG-1	14	ND	ND	0.018	ND
BLG-2	14	ND	ND	0.008	ND
LMB-1	28		ND	ND	ND
LMB-2	28		ND	ND	TR
LMB-3	28			ND	
CCF-1	28		ND		ND
CCF-2	28		ND	ND	ND
CCF-3	28		ND	ND	ND
BLG	28		ND	ND	ND
LMB	56		ND	ND	ND
CCF	56		ND	ND	ND
BLG	56		ND	ND	ND
LMB	84		ND	ND	
CCF	84		ND		ND
BLG	84		ND	ND	
LMB	112				ND
CCF	112		ND	ND	ND
BLG	112		ND	ND	ND
LMB	140		ND	ND	ND
CCF	140		ND		ND
BLG	140		ND		ND

(Continued next page)

TABLE 3 (cont'd). *Residues of DMA-2,4-D in fish from ponds treated with 2,4-D, 1971*

Species and fish number	Days after treatment	Residues (mg/kg) in fish treated at— 0 kg/ha.	2.24 kg/ha.	4.48 kg/ha.	8.96 kg/ha.
GEORGIA					
LMB	0	ND			
CCF-1	0	ND			
CCF-2	0	ND			
BLG	0	ND			
LMB	1		ND	0.014	0.022
CCF	1	ND	ND	0.043	0.008
BLG	1	ND	ND	0.024	0.044
LMB-1	3	ND	ND	ND	ND
LMB-2	3			ND	ND
LMB-3	3			ND	
CCF-1	3	ND	ND	ND	ND
CCF-2	3	ND	ND	ND	ND
BLG	3		ND	ND	ND
LMB-1	7	ND	ND	ND	ND
LMB-2	7			ND	ND
LMB-3	7				ND
CCF-1	7	ND	ND	ND	ND
CCF-2	7			ND	
BLG	7	ND	ND	ND	ND
LMB-1	14	ND		ND	ND
LMB-2	14			ND	ND
LMB-3	14			ND	ND
CCF-1	14	ND		ND	0.012
CCF-2	14			ND	
CCF-3	14			ND	
BLG-1	14	ND		ND	0.075
BLG-2	14			ND	ND
BLG-3	14			ND	ND
LMB-1	28	ND	ND	ND	0.010
LMB-2	28	ND	ND	ND	0.005
LMB-3	28	ND	ND		
LMB-4	28	ND			
CCF-1	28	ND	ND	ND	ND
CCF-2	28		ND	ND	
CCF-3	28		ND	ND	
BLG	28	ND	ND	ND	ND
LMB-1	56	ND	ND	ND	ND
LMB-2	56	ND	ND	ND	
CCF	56	ND	ND	ND	ND
BLG	56	ND	ND	ND	ND
LMB	84	ND	ND	ND	ND
CCF	84	ND	ND	ND	
BLG-1	84			ND	ND
BLG-2	84				TR
LMB-1	112	ND	ND	ND	ND
LMB-2	112			ND	
CCF	112	ND	ND	ND	ND
BLG	112	ND	ND	ND	ND
LMB-1	140	ND	ND	ND	ND
LMB-2	140	ND	ND		
LMB-3	140	ND			
LMB-4	140			ND	
LMB-5	140			ND	
CCF-1	140		ND	ND	ND
CCF-2	140		ND	ND	
BLG-1	140		ND	ND	ND
BLG-2	140			ND	
BLG-3	140			ND	
BLG-4	140				ND
BLG-5	140				ND
MISSOURI					
CCF	1			ND	ND
BLG	1			ND	ND
LMB	1			ND	ND
CCF	7				ND
BLG	7				ND
LMB	7				ND
CCF	14				ND
BLG	14				ND
LMB	14				ND
CCF-1	14		ND		
CCF-2	14		ND		
CCF-3	14		ND		
CCF-4	14		ND		
CCF-5	14		ND		
BLG-1	14		ND		
BLG-2	14		ND		
BLG-3	14		ND		

Species and fish number	Days after treatment	Residues (mg/kg) in fish treated at— 0 kg/ha.	2.24 kg/ha.	4.48 kg/ha.	8.96 kg/ha.
MISSOURI, cont'd					
BLG-4	14		ND		
BLG-5	14		ND		
LMB-1	14		ND		
LMB-2	14		ND		
LMB-3	14		ND		
LMB-4	14		ND		
LMB-5	14		ND		
CCF	28				ND
BLG	28				ND
LMB	28				ND
CCF-1	28				ND
CCF-2	28				ND
CCF-3	28				ND
CCF-4	28				ND
CCF-5	28				ND
BLG-1	28				TR
BLG-2	28				TR
BLG-3	28				TR
BLG-4	28				TR
BLG-5	28				TR
LMB-1	28				ND
LMB-2	28				TR
LMB-3	28				ND
LMB-4	28				ND
LMB-5	28				ND
CCF	56				ND
BLG	56				ND
LMB	56				ND

NOTE: LMB = largemouth bass, CCF = channel catfish, BLG = bluegills.
ND = not detectable.
TR = trace (less than 0.005 mg/kg).
Blank = no sample analyzed.

Discussion

WATER

Residues in Missouri pond waters were more persistent than were Florida and Georgia residues. This may have been caused partly by cooler water temperatures in the Missouri ponds which may have slowed the biological decomposition. For example, the median water temperature (shallow, medium, deep) in the three Missouri ponds averaged 26.6°C from the day of treatment to 28 days thereafter, whereas average temperatures in the Georgia and Florida ponds were 28.0°C and 30.9°C, respectively. Also, the Missouri ponds had been sprayed on the assumption that they were either 0.1 or 0.25 acres in area. However, when morphometric measurements were made, it was found that their areas in acres were as follow: Pond 9, 0.073; Pond 28, 0.162; and Pond 26, 0.140. Hence the initial concentration of 2,4-D (mg 2,4-D/liter water) in Missouri ponds was almost twice that in Florida and Georgia ponds (Table 1).

Residual persistence of the herbicide in Missouri ponds may also be attributed to direct application of the herbicide to the water surface. Because no waterhyacinth was present, there was little surface biomass to absorb or adsorb and degrade the chemical.

Wojtalik et al. (*2*) reported residues greater than 0.02 mg/liter at only 2 of 19 stations 4 weeks after treatment at 20 or 40 lb a.e./acre DMA-2,4-D. Aly and Faust (*12*) reported a photodecomposition loss of 50 percent of a sodium salt of 2,4-D in 50 minutes at pH 7. Hence the disappearance of DMA-2,4-D from water is rapid compared to the herbicide dichlobenil, 2,6-Dichlorobenzonitrile, which had a reported residue of 0.05 mg/liter 85 days after treatment at 0.55 mg/liter, and the herbicide fenac, 2,3,6-Trichlorophenylacetic acid, which had a reported residue of 0.77 mg/liter 85 days after treatment at 1.56 mg/liter (*13*).

MUD

The dissipation of 2,4-D from mud was quite rapid compared to dissipation of fenac and dichlobenil, which have been reported at 0.06 and 0.12 mg/kg, respectively, 160 days after treatment (*13*). Presumably, the dissipation of 2,4-D from mud was caused in great measure by microbiological degradation. At least 11 species of bacteria and two actinomycetes are known to degrade 2,4-D (*14*).

FISH

Chemicals can impart an off-taste or even toxicity to fish consumed by humans; hence it is imperative that residue levels in fish of any pesticide applied to aquatic environments be as low as possible. At this writing, no tolerance levels have been set for residues of DMA-2,4-D in fish.

Only 7 percent of the fish analyzed 28 days or more after treatment contained detectable 2,4-D residues and only 1 percent (one fish) of those analyzed 56 days or more after treatment contained detectable residues (Table 3). Thus if tolerance levels are based on the level of parent compound only, it would appear that fish could be consumed 1 month after treatment. However, degradation products were present in fish for as long as 84 days after treatment in plastic pools (*11*). Hence the presence of these unidentified products also should be considered.

Little, if anything, is presently known about the direct effects of herbicides on fish reproduction. However, reproduction of bluegills occurred in two Florida ponds, Willow and 11-T, during the experiment. Growth and development of the fry appeared to be normal. At no time was there any evidence of fish mortality caused by the herbicidal application.

Pesticides usually biomagnify through the food chain. Authors found that fish absorbed little 2,4-D when fed radio-labelled herbicide (*11*). Also, Wojtalik et al. (*2*) reported no harmful effects or accumulation in zooplankton, phytoplankton, or macroinvertebrates in water treated at 20 or 40 lb a.e./acre. Hence there is little danger of biomagnification of 2,4-D in contrast to the chlorinated hydrocarbon pesticides (*15*). However, when fish were exposed to ^{14}C-DMA-2,4-D in water, radioactive compounds were ubiquitous in all tissues examined (*11*). Therefore, the identity and potential toxicity of these compounds must not be overlooked. It also has been reported that although fish absorbed more 2,4-D at pH 6 than at pH 9, they retained more of the parent compound at a basic pH (*11*). Thus, aquatic applications of 2,4-D probably should be avoided under conditions of high pH.

Although fish can be killed by high concentrations of DMA-2,4-D, the reported TL_m at 96 hours (*11*) is high enough that even a tenfold error in application rate would not decimate a fish population. In the same study, death of fish from the herbicide was found not to result from a single, specific factor such as a carcinogenic effect; it undoubtedly resulted from a plethora of effects on carbohydrate metabolism. On the basis of this study, then, waterhyacinth can be controlled by judicious application of DMA-2,4-D at recommended rates without concomitantly high residue levels in fish, water, or hydrosol.

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RESIDUES IN FOOD AND FEED

Chlorinated Hydrocarbon Insecticide Residues in Feed and Carcasses of Feedlot Cattle, Texas—1972[1]

Donald E. Clark, Harry E. Smalley, H. R. Crookshank, and Florence M. Farr

ABSTRACT

Residues of several chlorinated insecticides in fat samples from calves that had been on controlled feed for 112 days were compared with residues in their feed. Both feed and fat samples contained lindane, dieldrin, DDT, DDE, and DDD. Except for dieldrin, each insecticide was found in higher levels in fat than in feed. Addition of ammonium sulfate and ammonium chloride as sources of nonprotein-nitrogen did not affect accumulation of residues. Average residue levels found in composite feed samples were: lindane, 0.002 ppm; DDE, 0.022 ppm; dieldrin, 0.029 ppm; DDD, 0.032 ppm; and DDT, 0.083 ppm. Average residue levels found in the fat were: lindane, 0.026; DDE, 0.261 ppm; dieldrin, 0.030 ppm; DDD, 0.101 ppm; and DDT, 0.292 ppm.

Introduction

The trend in beef production toward large feedlot operations has increased tremendously; the total number of cattle and calves in feedlots in the United States increased from about 7 million head in 1971 to 8.8 million head in May 1972 (*1*). In response to demands of large feedlot operations, the feed grain industry utilizes the services of professional livestock nutritionists and computer analysis to formulate rations on the basis of least-cost. Thus, ration components and amounts may change according to price but the ration remains constant in nutritional value. Rations are generally composed of high-energy, low-roughage sorghum grain with supplements of alfalfa meal, corn chops, or rice hulls for roughage. Premixed supplements containing a number of essential ingredients such as calcium, phosphorus, salt, trace minerals, antibiotics, and vitamins are also included. The complete ration is mixed and fed, free choice, to the cattle. Little or no information is provided about the origin of the feed components and there is very little pesticide residue monitoring of either the components or the mixed feed.

Many reports dealing with pesticide residues in specific forage crops have been published (*2-7*). Annual reports are made by Government regulatory agencies on pesticide residues in total human diet samples, individual items of food or crops at their origin, and meat and poultry samples taken at numerous slaughterhouses throughout the Nation (*8*). Present investigations were undertaken to determine occurrence and concentration of chlorinated insecticide residues in composite samples of a commercial feedlot ration as it was offered to animals, and occurrence and residue levels of these insecticides in visceral fat of animals at slaughter.

Parallel studies on the use of two levels of both ammonium sulfate and ammonium chloride as sources of supplemental nitrogen were conducted. Ammonium chloride acts as a diuretic and urine acidifier and controls urinary calculi in sheep and cattle (*9,10*). Studies were undertaken to determine whether the diuretic effect, under practical feedlot conditions, would result in increased excretion and thus decreased pesticide residues in fat samples.

Materials and Methods

The feedlot used in our 1972 study was located near Midland, Tex. Diets of experimental feed were mixed in a mill located on the premises, then distributed by truck to feed bunkers arranged on the periphery of each pen. Feed was mixed and animals were maintained under standard feedlot conditions.

Some 450 calves, purchased commercially and weighing approximately 280 kg (600 lb) each, were used in

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these studies. So far as authors know, none of the calves had been exposed to pesticides either before purchase or in the feedlot, except for possible residues incidental in their feed. Cattle were separated into five groups, examined to insure good health, identified, and kept in separate pens. Calves were randomly and evenly allotted to test groups; weights and feed consumption were recorded monthly (*11*).

The control diet (group I) was essentially the same as that used by the feedlot, but ammonium salts were excluded. In keeping with existing clearances for ammonium chloride set by the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare, no tetracyclines or growth hormones were included in the ration. Test diets for all five groups had equal levels of crude protein equivalent from nonprotein-nitrogen, and equal caloric values. Group II animals were fed approximately 20 g ammonium chloride per head per day; this is the level used to control calculi formation in the renal system of cattle (*9*). The group III ration supplied approximately 85 g ammonium chloride per head per day. Animals in groups IV and V were provided diets supplying approximately 25 and 100 g ammonium sulfate per animal per day. These dosages were established to provide the same amount of supplemental nitrogen as was provided groups I and II by ammonium chloride.

Samples of feed were taken after distribution to feed pens. Each day's mix was considered separately; thus random samples were taken twice weekly throughout the study. At each sampling, a cup of mixed feed was removed from both ends and the middle of each trough and the three cupfuls were combined to form a composite sample. The composite samples of each test group were combined and thoroughly mixed, and aliquots were taken for analysis.

Cattle were on feed for 112 days. They were then marketed and slaughtered in a commercial packing plant operating under the inspection of the U.S. Department of Agriculture (USDA). Lot integrity was maintained until final disposition of carcasses. After inspection of carcasses and viscera, samples of omental fat were taken from 50 animals, 10 randomly selected from each of the 5 lots. Samples were immediately frozen for later analysis.

Chlorinated insecticide residues were determined by electron-capture/gas-liquid chromatography (EC/GLC). Samples were prepared for analysis using a modified method of the procedures developed by Kadoum (*12,13*). Fat and feed samples were extracted by homogenization with hexane in a VirTis-23 homogenizer. Fat samples of 250 mg were cut into small cubes and placed in 5-ml homogenizing flasks; then 4.0 ml pesticide-quality hexane and about 1.5 g anhydrous sodium sulfate were added. Contents of the flasks were then homogenized for 10 minutes at medium speed. Feed samples of 0.5 to 2.0 g were similarly extracted with 10-20 ml hexane.

Sample cleanup was accomplished on silica gel elution columns. Prior to use, the absorbent, Woelm Activity Grade I silica gel was washed sequentially with methanol, benzene, and hexane; it was dried, first in air, then overnight in a 250° C oven. This process was necessary to eliminate the appearance of gas chromatographic background peaks. Hexane-washed distilled water was added to the dried silica gel for deactivation to a water content of 4.6 percent. Deactivated silica gel was stored in a glass-stoppered bottle until elution columns were prepared just before use. Glass columns 200 by 6 mm were plugged at the bottom with glass wool; 10 cm deactivated silica gel and 1 cm anhydrous sodium sulfate were placed in the column. The absorbent was wetted with 10 ml hexane just before addition of the sample. As soon as the hexane had percolated into the column, aliquots of either 1 ml fat extract or 2 ml feed extract were added. Pesticides were eluted with 1:1 benzene: hexane until a volume of 15 ml of the eluate had been collected. The solvent was removed by evaporation to 0.5 ml at 40°C under a gentle stream of dry nitrogen. The volume was then brought up to 1.0 ml with hexane. Appropriate aliquots were taken for GLC analysis.

Conditions and operating parameters for GLC analysis were as follows:

Instrument: Varian 2100-40 gas chromatograph
Detector: Ni-63; 225°C
Column: 6-ft.-by-¼-in. glass column packed with 1.5 percent OV-17 + 1.9 percent QF-1 on Supelco HD, 80/100 mesh; 195°C
Injector temperature: 225°C
Carrier gas: prepurified nitrogen, 18 ml/min at 50 psi
Attenuation: 4
Range: 1×10^{-10} amps/mv
Sensitivity: 15 percent full-scale (1 mv) response to 5 pg aldrin
Retention: aldrin—2.77 minutes

Insecticide peaks were identified by comparing their retention times and peak geometry with those resulting from addition of known amounts of insecticides to control fat extracts. Additional confirmatory tests were not conducted.

Known amounts of insecticides were added to control samples before extraction in order to determine analytical efficiency.

Results and Discussion

The extraction procedure employed in this study gave satisfactory recoveries from fat tissue of each of the chlorinated insecticides and derivatives studied (Table 1). Gas chromatograms from extracts of control samples were free from interfering peaks at retention times of insecticides in question.

TABLE 1. *Recovery of chlorinated insecticides from fortified fat samples*

	Lindane				Heptachlor Epoxide				Dieldrin				*p,p'*-DDE				*p,p'*-DDD				*p,p'*-DDT			
Quantity, ppm Added	0.020	0.040	0.200	0.400	0.030	0.060	0.300	0.600	0.040	0.080	0.400	0.800	0.040	0.080	0.400	0.800	0.100	0.200	1.000	2.000	0.150	0.300	1.500	3.000
Recovered	0.019	0.036	0.184	0.356	0.027	0.059	0.291	0.582	0.039	0.066	0.040	0.672	0.038	0.073	0.376	0.800	0.102	0.152	0.860	1.680	0.152	0.273	1.385	2.550
Percent recovery	97	90	92	89	90[1]	99	97	97	98	83	100	84	95	92	94	100	102	76	86	84	106	91	89	85

NOTE: All recoveries are average of two replications.
[1] Single sample.

TABLE 2. *Chlorinated insecticide residues in commercial feed and fat of feedlot cattle after consumption of feed with and without ammonium salts added, Texas—1972*

	Residues, Cattle Fat					
Insecticide	Group I Untreated Controls	Group II NH_4Cl 20 g/day/animal	Group III NH_4Cl 85 g/day/animal	Group IV $(NH_4)_2SO_4$ 25 g/head/day	Group V $(NH_4)_2SO_4$ 100 g/head/day	Residues, Feed
Lindane	0.029 ± 0.007	0.025 ± 0.004	0.028 ± 0.009	0.032 ± 0.005	0.014 ± 0.010[1]	0.002 ± 0.001
DDE	0.244 ± 0.037	0.274 ± 0.146	0.227 ± 0.076	0.295 ± 0.110	0.236 ± 0.094	0.022 ± 0.004
Dieldrin	0.033 ± 0.017	0.022 ± 0.027	0.033 ± 0.027	0.044 ± 0.052	0.030 ± 0.023	0.029 ± 0.007
DDD	0.095 ± 0.040	0.121 ± 0.093	0.099 ± 0.033	0.139 ± 0.051	0.086 ± 0.054	0.032 ± 0.008
DDT	0.340 ± 0.096	0.324 ± 0.256	0.430 ± 0.106	0.518 ± 0.104	0.352 ± 0.212	0.083 ± 0.017

NOTE: All residues reported in ppm.
Values reported as mean ± standard deviation.
Each group represents 10 animals.
[1] Significant, $p = 0.05$

Gas chromatographic analysis indicated that lindane, dieldrin, DDE, DDD, and DDT were present in both feed samples and fat from slaughtered animals (Table 2). Average residues in fat were lindane, 0.026 ppm; DDE, 0.261 ppm; dieldrin, 0.030 ppm; DDD, 0.101 ppm; and DDT, 0.292 ppm. Heptachlor epoxide residues were not detected in any samples analyzed. Values in Table 2 represent means and standard deviations (uncorrected for recovery) calculated from 10 fat samples and 5 feed samples in each group. Residues reported herein are based on adipose tissue as removed from the animals. Such tissue contains 80-85 percent extractable fat.

No evidence of toxicity was observed in any of the cattle; this was expected, considering the very low levels of pesticide consumed. However, observations were made during the test for possible enhancement of toxicity from the addition of ammonium salts to the diet. All animals gained weight normally during the test and showed no evidence of toxic conditions at slaughter.

With the exception of the insecticide dieldrin, all residues found in fat samples were much higher than residues observed in feed. Magnification of this type is well documented and is to be expected. Dieldrin residues present in the fat of each of the animal groups very closely approximate levels found in the diet. The Duncan Multiple Range Test (*14*), used to determine significance of differences in residues resulting from the test diets, indicated that with the exception of lindane residues in the fat of animals receiving high ammonium sulfate levels, pesticide residue levels were not affected by addition of either ammonium chloride or ammonium sulfate to the diet. Fat samples from animals receiving 100 g/head/day ammonium sulfate had lindane residues significantly lower than those from other treated animals or from control animals.

Conclusions

Low residue levels of five chlorinated insecticides, lindane, dieldrin, DDE, DDD, and DDT, were found in feed and fat of west Texas feedlot cattle. Levels in feed were less than 0.1 ppm; those in fat were less than 0.5 ppm.

Except for decreased lindane residues in cattle receiving 100 g ammonium sulfate daily, chlorinated pesticide residues were not affected by ammonium chloride or ammonium sulfate when ammonium compounds were used as nitrogen supplements.

No ill effects resulting from interaction of pesticides and ammonium compounds were observed in any of the groups.

The analytical method was sensitive and efficient for analysis of fat samples for low levels of multiple chlorinated insecticide residues.

Acknowledgments

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GENERAL

DDT and Dieldrin in Watersheds Draining the Tobacco Belt of Southern Ontario

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ABSTRACT

Production of flue-cured tobacco in Ontario is concentrated mainly in four watersheds adjacent to Norfolk County on the north shore of Lake Erie, where tobacco is raised on a sandy loam soil using a 2- to 3-year rotation with cereals and corn. In 1961-69 an average amount of 4.4 kg/ha. DDT was used to control soil and foliar insects attacking tobacco. In 1970-71, DDT was restricted to the control of soil insects only; applications averaged 1.7 kg/ha. Residue data from soil samples collected in 1971 indicated total levels of 325,000 kg ΣDDT and 14,000 kg dieldrin in the entire tobacco-growing area. These residues represented 37 percent of the total DDT application to the area since 1961, and were calculated to agree with the half-life disappearance of 3-4 years.

Residue analysis of the tobacco crop in 1971 (42 million kg cured leaf) revealed that 52.6 kg ΣDDT and 1.97 kg dieldrin had been removed from the soil. This represents 0.18 percent of the DDT applied in 1971 or 0.03 percent of the ΣDDT and dieldrin resident in the soil the same year. Corn silage and hay were calculated to contain 38.3 kg DDT and 0.76 kg dieldrin, but these residues remained in the watershed because the crops were used as feed for livestock. Animal products leaving the watershed in 1971 amounted to 54 million kg milk and 6 million kg meat which contained only 0.95 kg ΣDDT and 156 g dieldrin.

Total water discharging into Lake Erie from the four watersheds was calculated to carry 12.6 kg ΣDDT and 0.86 kg dieldrin in the water and on suspended sediments, representing 0.002 percent and 0.003 percent, respectively, of the ΣDDT and dieldrin resident in the four watershed soils.

Fish species caught in Big and Dedrich Creeks contained residues 4 to 15 times higher than those of species from Long Point Bay and Lake Erie, reflecting the rapid dilution of the creek waters within 3 miles of the mouths of these creeks. Lowest pesticide concentrations in fish were about the same as the concentrations in water-suspended sediments. Magnification observed from water to the highest concentrations in large fish was approximately 10^5; however, the tissue residue did not exceed the 5 ppm tolerance established in fish.

Residues in air appeared to be the result of either spray drift or airborne soil particles rather than volatilization from the soil.

Introduction

Production of flue-cured tobacco in Ontario is concentrated in a few counties along the north shore of Lake Erie. Forty-six percent of this cropping area is located in the four watersheds of Big Creek, Big Otter Creek, Dedrich Creek, and Nanticoke Creek, which are centered around Norfolk County (Fig. 1). The total area of these four watersheds is 190,340 hectares (ha.) (Table 1). The land is flat or gently undulating and the soil is a deep sandy loam derived from alluvial deposits. Agricultural production occupies 80 percent (152,610 ha.) of the watershed area and tobacco production occupies 10-15 percent of this agricultural land (*1*). Corn and grain, especially rye, are alternated with tobacco in a 2- to 3-year crop rotation.

The dark-sided cutworm *(Euxoma messoria)* has been a serious pest to tobacco production of the area since 1961. This species has shown a high tolerance to cyclodiene insecticides (*2*); thus DDT has been used, instead, as an effective early-spring treatment applied to the rye cover crop before plow-down or to soil before planting tobacco (*3,4*). DDT has also been used to control leaf-eating insects during the season. As a result

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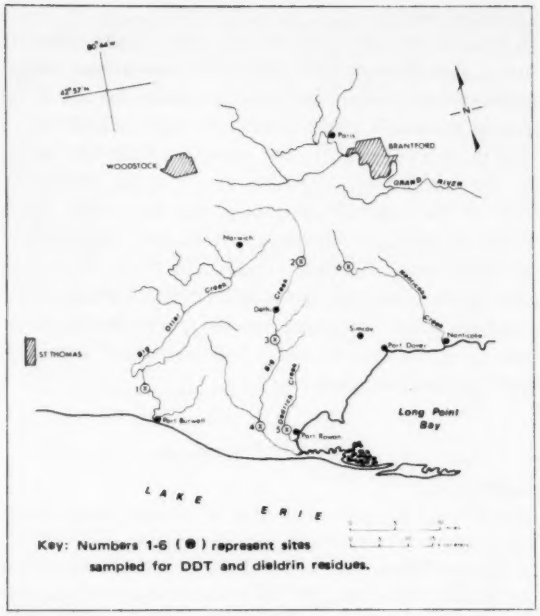

FIGURE 1. *Four watersheds draining tobacco belt of southern Ontario, Canada*

of these treatments, Harris and Sans have reported residues between 3 and 4.5 ppm ΣDDT in tobacco soils of Ontario (*5*). Residues increased from 3.1 ppm in 1964 to 4.6 ppm in 1966, and declined slightly in 1969 to 3.4 ppm.

The pathway of DDT and its metabolites into the aquatic environment on soil particles is well documented for many parts of the world. Residues found in the water, sediment, and fish in this area have been reported by Miles and Harris, and Frank et al. According to Miles and Harris, highest concentrations were 67 parts per trillion (ppt) in water, 441 parts per billion (ppb) in mud, and 1.0 parts per million (ppm) in fish (*6*). Frank et al. reported a mean of 91 ppb ΣDDT in sediments and a residue range of 0.03 to 3.86 ppm in fish depending on the species and its position in the aquatic food chain (*7*).

Miles and Harris noted a correlation between rainfall and the concentration of insecticides in Big Creek, and reported that this creek carried 50 g DDT per week into Lake Erie in 1970 (*6*). Although the largest quantity of DDT was used in this area, residues in river sediment were no higher than those in recreational areas where only small quantities were used for spraying mosquitoes (*7*). This was especially evident on the Precambrian Shield where rock was bare or only thinly covered with soil, and DDT readily found its way to lakes and streams following rain.

Joint action by the Ontario Ministries of the Environment and of Agriculture and Food restricted DDT to three permit uses in 1970. Included was a use for cutworm control on tobacco which permitted application of 1.7 kg/ha. to a rye cover crop at plow-down or a soil treatment before planting. Between 85 and 90 percent of the DDT applied under permit in Ontario was used in tobacco production and about 40 percent, 28,970 kg, was used in the study area (Table 1).

Most of this study was carried out in 1971 within the boundary of the specified watersheds with the intention of drawing up a balance sheet on the use and dispersal of DDT. Samples were collected from the physical environment, agricultural products, and aquatic biota.

Field Procedures

PHYSICAL ENVIRONMENT

Authors selected six sites on the four watersheds drained by Big Creek, Big Otter Creek, Dedrich Creek, and Nanticoke Creek for regular sampling; four of these, 1, 4, 5, and 6, were so located that 96, 87, 100, and 81 percent of the tobacco acreage, respectively, were upstream from the site.

Sample sites 1, 4, and 5 on Big Otter, Big, and Dedrich Creeks, respectively, were only a short distance upstream from flow gauge meters; they were located on sections of each creek where no major inflow of water occurred between any two creeks. Site 6 on Nanticoke Creek was in the tobacco belt; the flow meter was located downstream at the town of Nanticoke. Water passing the gauge was collected occasionally during the season and compared with water from site 6.

TABLE 1. *Land acreage, water discharge, and DDT use by watershed, southern Ontario—1971*

WATERWAY	WATERSHED AREA, ha.	WATER DISCHARGE ($\times 10^6$ m^3)	AGRICULTURAL AREA, ha.	TOBACCO, ha.	DDT PURCHASED,[1] kg
Big Otter Creek	79,965	165.0	71,430	6,156	11,175
Big Creek	81,815	143.0	57,830	8,562	15,288
Dedrich Creek	8,650	12.9	6,190	512	927
Nanticoke Creek	19,910	33.4	17,160	870	1,580
TOTAL	190,340	354.3	152,610	16,100	28,970

[1] DDT purchased by permit.

Creek water samples of 4.5 liters were collected weekly throughout the season from all six sites. Samples were taken from the center of the streams between the surface and the water-sediment interphase. Although most waters were clear, all were filtered through Whatman No. 1 filter paper to remove suspended material. Water was quite clear and colorless following this procedure; deposits were too small to analyze.

Creek sediment samples weighing 450 g were collected weekly from the same locations by scraping along the creekbed and skimming up surface and mobile sediments. Water and sediments were also collected off the mouths of Big and Dedrich Creeks and from Inner Long Point Bay during the spring and late summer.

Soils were collected during the spring and fall from one woodlot and several rye and tobacco fields on six to eight farms around each of the six sites. Rye and tobacco soils were sampled from surface to plow-depth.

An air monitor was set up in the middle of the tobacco belt close to site 3 on Big Creek; airborne organics were extracted into acetonitrile on 33 days (24-hr periods) between May 13 and August 31, 1971. Between 3,540 and 3,960 liters of air were passed through the acetonitrile in a 24-hour period.

AGRICULTURAL PRODUCTS

Flue-cured tobacco was sampled at the end of the 1971 season from 20 farms in the four watersheds. Composites of 500 g were obtained from each of the five primings by sampling several bales on each farm. Composite milk samples of 1.1 liters were obtained from 26 bulk transporters that collected the total volume of milk produced in the four watersheds. Samples were taken directly from the tanker following mechanical mixing. Collections were made over a 2-week period in early summer 1971. Composite beef fat samples of 100 g were collected from abattoirs where animals were known to have come from the study area. A 1-inch cube of fat was removed from the backs of up to 10 carcasses to procure one sample.

Samples of corn silage, hay, drinking water, milk, and beef were collected from farms around the six creek sites in October 1971. Silage samples of 25 kg were taken during the normal course of unloading and feeding; composite hay samples of 10 kg were obtained from a minimum of 10 bales in storage; water samples of 1 liter were collected from farm ponds and wells; and milk samples of 0.6 liter were taken from holding tanks on farms where silage or hay was obtained. Beef fat was obtained from farms where animals were going for slaughter.

AQUATIC BIOTA

A total of 289 fish of 24 species were caught either by net or line between 1969 and 1972. Researchers caught eight species predominantly in the creeks and ten in the bay or the lake; six species were common to both creeks and the lake (Table 2). The majority of the fish caught in the creeks were from Big Creek or its tributaries; only a few were obtained from the Big Otter. Those from Dedrich and Nanticoke Creeks were caught close to the mouths and were common to Long Point Bay. All fish were weighed and measured before evisceration. Analyses were conducted on the puree produced by homogenizing the eviscerated fish. Individual fish which were analyzed weighed over 25 g.

Analytical Procedures

EXTRACTIONS

Researchers partitioned 1 liter of filtered water twice by shaking it vigorously for 60 seconds with 50 ml dichloromethane. Extracts of dichloromethane were dried by passing them through anhydrous sodium sulfate and evaporating just to dryness with rotary vacuum at 45° C. Residues were redissolved in 10 ml hexane. Soil and sediment samples were air dried and ground to a fine consistency. A 25-g sample was moistened with 4 ml water and allowed to stand for 12 hours. Soil was extracted with 250 ml 1:1 acetone:hexane (v/v) by shaking briskly on a wrist-action shaker for 2 hours. A 100-ml aliquot was removed by filtering and shaking with 300 ml 2 percent aqueous sodium chloride for 60 seconds. The hexane extract was dried by passage through anhydrous sodium sulfate; it was evaporated to 5-10 ml by rotary vacuum at 45° C.

The acetonitrile solution from the air impinger was reduced to approximately 100 ml by rotary vacuum at 75° C. The solution was partitioned by shaking vigorously, first with 100 ml hexane for 60 seconds, then with 500 ml 2 percent aqueous sodium chloride for 30 seconds. The hexane extract was dried by filtration through anhydrous sodium sulfate; it was reduced to 5-10 ml by rotary vacuum at 45° C.

Hay and silage samples of 25 g dry weight were extracted by blending them with 250 ml 2:1 acetonitrile: water. A 100-ml aliquot was filtered off and partitioned into 100 ml hexane. The extract was concentrated to 5-10 ml by rotary vacuum. Moisture content of the silage and hay was determined by drying at 80° C.

Butterfat was separated from milk samples with a detergent reagent which had been prepared from 200 g sodium tetraphosphate and 24 ml Triton X-100 in 1 liter distilled water. Butterfat was separated in a water bath at 95°-100° C with 100 ml milk and 100 ml detergent.

TABLE 2. *Accumulations of DDT, its metabolites, and dieldrin in several fish species by weight class*

Species	Location	Weight Class, g	No. Fish Analyzed	Average Weight, g	ΣDDT Tissue, ppm	ΣDDT Fat, ppm	ΣDDT Fish, μg	Dieldrin Tissue, ppm	Dieldrin Fat, ppm	Dieldrin Fish, μg
Watershed Creeks and Lake Erie										
Clupeiformes										
Cyprinidae										
Carp (*Cyprinus carpio*)	Creek	All	1	2460	0.45	5.47	1107	0.04	0.49	98.4
	Lake	All	1	577	0.05	3.13	28.9	0.005	0.31	2.89
Perciformes										
Centrarchidae										
Rock bass (*Ambloplites rupestris*)	Creek	All	5	90	0.76	20.8	82.9	0.04	1.05	3.38
	Lake	All	13	147	0.076	4.15	11.8	0.003	0.16	0.25
Bluegill (*Lepomis macrochirus*)	Creek	All	2	180	0.075	1.98	12.2	0.005	0.186	1.15
	Lake	All	4	209	0.021	4.07	3.33	0.001	0.138	0.126
Pumpkinseed (*Lepomis gibbosus*)	Creek	All	7	82	0.60	13.0	23.2	0.024	0.48	1.32
	Lake	All	5	98	0.041	3.15	4.02	0.005	0.37	0.48
Siluriformes										
Ictaluridae										
Brown bullhead (*Ictaluris nebulosus*)	Creek	All	3	301	0.430	60.0	86.3	0.018	2.68	3.16
	Lake	All	3	137	0.039	16.4	4.17	0.002	0.72	0.23
Amiiformes										
Amiidae										
Bowfin (*Amia calva*)	Mouth	All	1	765	0.03	2.24	24.5	0.004	0.28	3.06
Watershed Creeks										
Cypriniformes										
Catostomidae										
White sucker (*Catostomus commersoni*)		0-100	2	6	0.010	18.5	0.07	0.004	6.00	0.02
		101-200	4	153	0.405	14.9	25.3	0.019	2.19	2.87
		over 200	2	472	0.050	51.2	30.4	0.004	1.06	1.89
Cyprinidae										
Creek chub (*Semotilus atromaculatus*)		All	5	72	0.678	16.0	33.9	0.036	1.17	1.96
Blacknose dace (*Rhinichthys atratulus*)		All	16	2.2	1.07	13.8	1.50	0.080	1.23	0.12
Spottail shiners (*Notropis hudsonius*)		All	10	5.2	0.382	10.4	3.03	0.018	0.43	0.07
Perciformes										
Centrarchidae										
Largemouth bass (*Micropterus salmoides*)		All	2	153	3.86	51.2	740.0	0.190	4.92	34.9
Salmoniformes										
Salmonidae										
Brown trout (*Salmo trutta*)		All	6	122	1.44	30.7	164	0.047	1.02	5.9
Rainbow trout (*Salmo gairdneri*)		0-1000	4	270	1.43	38.6	229	0.050	1.44	7.6
		1001-2000	2	1415	1.08	18.1	1518	0.100	1.68	137
		2001-3000	2	2750	1.03	18.3	2828	0.070	1.24	193
		over 3000	1	6400	1.35	25.1	8640	0.070	1.30	448
Umbridae										
Central mudminnows (*Umbra limi*)		All	3	3.7	0.81	27.1	3.0	0.050	1.67	0.19
Long Point Bay and Lake Erie										
Clupeiformes										
Clupeidae										
Alewife (*Alosa pseudoharengus*)		All	8	102	0.25	1.13	26.2	ND	ND	ND
Cypriniformes										
Catostomidae										
Northern redhorse (*Moxostoma macrolepidotum*)		All	3	742	0.162	10.8	119	0.009	0.585	6.06
Perciformes										
Centrarchidae										
Smallmouth bass (*Micropterus dolomieui*)		0-150	9	87	0.12	5.3	10.3	0.005	0.24	0.47
		150-300	11	220	0.48	30.8	104.4	0.005	0.33	1.21
		300-450	8	373	0.54	26.4	194	0.012	0.40	4.66
		450-600	6	486	1.04	34.1	485	0.020	0.59	9.63
		over 600	3	1192	0.88	13.3	1241	0.013	0.25	11.6
Black crappie (*Pomoxis nigromaculatus*)		0-100	5	75	0.117	7.82	8.71	0.019	1.02	1.12
		100-200	2	152	0.174	15.6	26.3	0.006	0.50	0.85
Green sunfish (*Lepomis cyanellus*)		All	4	199	0.050	2.18	11.0	ND	ND	ND
Percidae										
Yellow perch (*Perca flavescens*)		0-50	6	47	0.102	3.33	4.69	0.024	0.87	1.12
		51-100	18	79	0.063	3.16	3.70	0.009	0.54	0.70
		101-150	15	126	0.081	7.84	10.13	0.003	0.29	0.36
		over 150	3	174	0.043	3.75	7.56	0.008	0.67	1.38
Sciaenidae										
Freshwater drum (*Aplodinotus grunniens*)		0-200	7	171	0.047	0.71	7.90	ND	ND	ND
		201-400	7	316	0.071	0.81	27.0	ND	ND	ND
		over 400	6	513	0.245	3.24	128	0.007	0.031	3.45
Serranidae										
White bass (*Morone chrysops*)		50-100	9	82	0.101	5.82	7.81	0.004	0.29	0.27
		101-150	19	118	0.122	5.70	14.9	0.007	0.44	0.90
		151-200	10	180	0.153	3.87	27.8	0.009	0.29	1.69
		over 200	4	394	0.470	5.69	229	0.013	0.16	6.52
Salmoniformes										
Osmeridae										
American smelt (*Osmerus mordax*)		0-20	4	16	0.13	7.4	2.0	0.009	0.52	0.14
		20-40	9	28	0.19	8.4	5.3	0.013	0.55	0.34
Salmonidae										
Coho salmon (*Oncorhychus kisutch*)		0-1000	7	622	1.14	18.0	829	0.016	0.24	10.2
		over 1000	2	2883	2.38	41.5	6652	0.004	0.07	11.5

Beef or beef fat containing significant amounts of connective tissue was extracted by soxhlet as described for fish tissues.

Eviscerated fish were ground into a puree and 10 g were mixed thoroughly with sodium sulfate and Ottawa sand. The mixture was exhaustively extracted by soxhlet over a period of 6 hours with 300 ml hexane. Solvent was removed by rotary-vacuum evaporation and the amount of lipids was determined gravimetrically.

COLUMN CLEANUP

Water, air, soil, sediment, hay, and silage extracts and up to a maximum of 1 g extractible fat or oil from milk, meat, and fish were quantitatively mixed with 25 g florisil (60/100 mesh) previously deactivated by equilibration with 5 percent water. A second 25-g portion of similarly deactivated florisil was first added to a chromatographic column, and the florisil, plus extract, was then added to form the upper half of the cleanup column. Columns were eluted with 300 ml 1:4 dichloromethane:hexane (v/v) at a rate of approximately 5 ml/min. (*8*). The eluate was evaporated just to dryness with rotary vacuum at 45° C and redissolved in hexane. Polychlorinated biphenyls (PCB's) were separated from the samples on a charcoal column before gas-liquid chromatographic determinations. PCB results will appear in a later report.

GAS CHROMATOGRAPHY

Qualitative and quantitative gas chromatographic determinations were carried out using the following parameters:

Columns:	152-cm-by-3.2-mm-OD Pyrex containing 4 percent SE-30 + 6 percent QF-1 on 80/100 Chromosorb W-AW preconditioned 72 hr at 225° C.
Detector:	Electron capture, either tritium or Ni-63.
Temperatures:	Injection block 225° C. Column 175° C isothermal. Detectors 200° C (tritium), 275° C (Ni-63).
Carrier Gas:	Nitrogen at 40 ml/min.

Limits of detection for all ingredients except DDT were 0.001 ppb in water, 0.001 ppm in soil, sediment, and plant tissue, and 0.005 ppm in extractible fat of milk, meat, and fish. For DDT the limit of detection was twice these levels.

CONFIRMATORY PROCEDURES

When concentrations permitted, qualitative DDT confirmations were carried out by thin-layer chromatography (TLC) using a 250-μ layer of silica gel developed with 1 percent chloroform in n-heptane and visualized with alkaline silver nitrate. Additional confirmation was achieved by removing the appropriate section of the TLC adsorbent and eluting it with a polar solvent for reexamination by gas chromatography. To form the respective dehydrochlorinated derivatives which were measured by gas chromatography (*9*), *p,p'*-DDT and *p,p'*-TDE were confirmed by treatment with alcoholic potassium hydroxide.

RECOVERY STUDIES

Recoveries of pesticides were checked periodically by direct fortification into the substrate with an acetone solution followed by extraction and cleanup as described above. Fortified milk samples were allowed to stand 24 hours prior to separation of butterfat; soil and sediment samples stood 1 week before extraction. The efficiency of the air sampler could not be practically determined. Recoveries from air samples are indicative only of the acetonitrile partitioning and cleanup operations.

Averaged percent recoveries were as follow:

	Water	Soil and Sediment	Milk, Beef, and Fish Fat
p,p'-DDE	98	91	96
p,p'-TDE	96	89	92
p,p'-DDT	94	87	91
o,p'-DDT	94	91	93
Dieldrin	96	86	89

Residues were determined on the basis of dried soil, sediment, silage, hay, tobacco, and the extractible fat of milk, meat, and fish. Moisture and fat contents were used in calculating quantities resident in animal populations of the watersheds. Results were not adjusted to include recovery percentages.

Results

DDT USE

Between 1961 and 1969 about 821,400 kg DDT were used on 183,200 ha. tobacco. Annual acreage varied from a high of 23,850 ha. in 1967 to a low of 13,680 ha. in 1964 (Table 3). Following the DDT restrictions of January 1, 1970, rates per hectare were reduced from about 4.4 to 1.7 kg/ha. and purchase was restricted to permitholders. In 1970 and 1971, 61,330 kg were applied to 33,280 ha. tobacco. Thus, over the 11-year period of 1961 through 1971, a total of 882,730 kg DDT was applied to 216,480 ha. tobacco in the four watersheds (Table 3).

SOILS

In the four watersheds 40,455 ha. of land is devoted to tobacco production in a 2- to 3-year rotation involving rye, corn, and wheat (Table 4). In 1971 about 16,100 ha. were devoted to tobacco; 24,355 ha. were cultivated for grain.

Rye fields sampled in April and May 1971 had not been cropped with tobacco since 1968 or 1969, but were to be planted late in May 1971. Mean ΣDDT concentrations in soil by watershed varied from 2.33 to 3.44 ppm (Table 4); dieldrin concentrations varied from 0.10 to 0.19 ppm. Authors calculated that a minimum of 173,700 kg DDT and 8,780 kg dieldrin could be resident in these soils.

TABLE 3. *Quantities of tobacco grown and DDT used, southern Ontario—1961-71*

Year	Tobacco Harvested, ha.[1]	DDT Applied, kg
1961	22,760	102,100
1962	21,700	97,300
1963	18,530	83,100
1964	13,680	61,300
1965	16,170	72,500
1966	21,880	98,100
1967	23,850	106,900
1968	22,730	101,900
1969	21,900	98,200
1961-69 (subtotal)	183,200	821,400
1970	17,180	32,360
1971	16,100	28,970
1970-71 (subtotal)	33,280	61,330
TOTAL	216,480	882,730

[1] Agricultural Statistics for Ontario, 1971. (Refer to Literature Cited, reference 1.)

TABLE 4. *ΣDDT and dieldrin in soils of tobacco fields and adjoining woodlots of four watersheds, southern Ontario—1961-71*

Watershed	Area, ha.	No. Samples	Concentration in Soil			Residues in Soil, kg	
			ΣDDT, ppm	DDE/TDE, %	Dieldrin, ppm	ΣDDT	Dieldrin
Soil: Rye, Wheat, and Corn Fields[1]							
Big Otter Creek	9,235	10	2.96	19.7	0.19	61,278	3,934
Big Creek	12,845	30	3.44	22.0	0.14	99,051	4,031
Dedrich Creek	765	10	3.20	18.0	0.10	5,487	171
Nanticoke Creek	1,510	10	2.33	21.8	0.19	7,884	643
Total	24,355	60				173,700	8,779
Mean			2.98	20.4[2]	0.16		
Soil: Tobacco Fields[3]							
Big Otter Creek	6,156	12	3.98	19.2	0.19	54,921	2,622
Big Creek	8,562	20	4.36	19.0	0.14	83,683	2,687
Dedrich Creek	512	8	3.95	19.3	0.19	4,522	218
Nanticoke Creek	870	10	3.88	24.8	0.10	7,571	195
Total	16,100	50				150,697	5,722
Mean			4.04	20.6[2]	0.16		
Total acreage in tobacco	40,455						
Soil: Woodlots							
Big Otter Creek	478	3	0.307	13.0	Trace	329	4.3
Big Creek	882	5	0.048	50.0	Trace	95	7.9
Dedrich Creek	61	2	0.074	73.0	Trace	10	0.5
Nanticoke Creek	101	2	0.024	33.0	Trace	5.4	0.9
Total	1,522	12				439.4	13.6
Mean			0.113	42.0[2]	Trace		
Grand Total	41,977	122				324,840	14,515

[1] Residue at end of rotation before planting tobacco.
[2] DDE represented 17.3 and 17.5 percent ΣDDT and TDE represented 3.1 percent ΣDDT; hence DDT represented 79.6 and 79.4 percent ΣDDT.
[3] Residue in soil after tobacco was harvested.

Tobacco fields were sampled in early October 1971, shortly after the crop had been harvested and stalks had been disked under. DDT had been applied in May at plow-down of the rye cover crop or just before planting tobacco. Concentrations of ΣDDT ranged from 3.88 to 4.36 ppm with a mean of 4.04 ppm (Table 4). Because the same fields were involved in rye and tobacco, increase in soil residues suggested that at least 2.35 kg/ha. DDT had been added during the growing season for the two crops. The amount of DDT resident in soil cropped with tobacco in 1971 was calculated at 150,700 kg. Dieldrin residues in soil remained unchanged from rye to tobacco fields and about 5,700 kg was calculated to be present in the tobacco acreage.

Composition of the ΣDDT in soil collected from rye and tobacco fields showed little difference. In both cases almost 80 percent was present as *o,p'*- and *p,p'*-DDT and only 20.4 and 20.6 percent was present as the two metabolites, DDE (17.1-17.3 percent) and TDE (3.1 percent).

A total of 1,522 ha. (0.8 percent of the four watersheds) is devoted to woodlots. Many of these woodlots are adjacent to or part of tobacco farms. Samples of woodlot soils were taken in both spring and fall. Because no woodlot had actually been sprayed, the mean residue of 0.113 ppm (Table 4) represents drift from either aircraft or ground applications, or else the accumulation of deposits from wind erosion. It was calculated that 440 kg ΣDDT and 14 kg dieldrin were resident in these woodlots. Composition of ΣDDT in these soils varied greatly from as little as 27 percent to as much as 87 percent *o,p'*- and *p,p'*-DDT.

A total of 324,840 kg ΣDDT was calculated to be present in the 41,977 ha. of land, including woodlots, involved in tobacco production. This amount represented almost 37 percent of the total estimated application of 882,730 kg over the 11-year period. Assuming a half-life of 3 and 4 years for ΣDDT, the predicted residue falls between 297,000 and 386,000 kg. These calculations support a half-life of 3 to 4 years for DDT in the light sandy loams observed in this study.

Total dieldrin residue amounted to 14,515 kg in 41,977 ha. of land; no estimate of use over the 11-year period was available. Use of aldrin and dieldrin in the 5 years preceding this study had been slight, largely because of the increasing preponderance of the dark-sided cutworm which exhibited a high tolerance to cyclodiene insecticides (*2*).

TOBACCO

The largest exported crop which removed organochlorine residues from the soil of the four watersheds was tobacco (Tables 4,5). The residue level of ΣDDT in cured tobacco leaf varied from farm to farm; mean residues declined from 2.52 ppm in sand leaves to 0.66 ppm in tips. The major portion of this ΣDDT was *o,p'*- and *p,p'*-DDT; 21-30 percent was DDE, and 4-9.5 percent was TDE. The amount of ΣDDT in the 1971 crop was calculated at 52.6 kg. If it were all derived from the 1971 application, this would represent 0.18 percent of the 28,970 kg applied. If it were derived from ΣDDT resident in tobacco soils during the growing season, it would represent only 0.035 percent.

Dieldrin residues were insignificant, declining from 0.09 ppm in the sand leaves to 0.02 ppm in the tips. The amount of dieldrin calculated to be present in the crop was 1.97 kg, representing 0.035 percent of that resident in the planted tobacco acreage.

SILAGE

Silage in the four watersheds is produced largely from corn which is often inserted in the tobacco-grain rotation in place of wheat for feeding dairy or beef cattle. In the four watersheds approximately 6,028 ha. corn produced 172.7 x 10^6 kg silage during 1971 (Table 6). The weighted mean of this silage crop contained 0.339 ppm ΣDDT and 0.005 ppm dieldrin on a dry-weight basis. Total quantity of ΣDDT in the whole crop was calculated at 34.2 kg and 536 g dieldrin.

Composition of DDT in the silage varied greatly from a high of 74 percent to a low of only 21 percent *o,p'*- and *p,p'*-DDT. Likewise, the *p,p'*-DDE content ranged from 22 to 77 percent; TDE content ranged from 2 to 6 percent. These findings suggest that residues resulted from spray drift where *o,p'*- and *p,p'*-DDT were high, and from soil uptake or soil contamination where DDE was high.

TABLE 5. *ΣDDT and dieldrin in cured tobacco leaf of four watersheds, southern Ontario—1971*

Priming (Leaves)	Cured Leaf [1] Production (× 1000 kg)	Content in Dried Cured Leaf, ppm		DDT Metabolites, %		Residues in Crop, kg	
		ΣDDT	Dieldrin	DDE	TDE	ΣDDT	Dieldrin
1 Sand	4,211	2.52	0.09	21.4	9.5	10.61	0.38
2 Seconds	5,894	2.08	0.07	23.1	7.7	12.26	0.41
3 Thirds	8,000	1.18	0.07	24.6	5.9	9.44	0.56
4 Fourths	13,893	0.98	0.03	29.6	4.1	13.62	0.42
5 Tips	10,104	0.66	0.02	28.8	4.5	6.67	0.20
Total	42,102					52.60	1.97
Mean		1.25	0.05	26.7	5.6		

[1] Records of Ontario Flue-Cured Tobacco Marketing Board, 1971. (Refer to Literature Cited, reference 15.)

TABLE 6. *ΣDDT in corn silage and hay of four watersheds, southern Ontario—1971*

Watershed	Crop Acreage, ha.	Crop Production (× 1000 kg)	Moisture, %	Residues in Dried Feed		Residues in Total Feed	
				ΣDDT, ppm	Dieldrin, ppm	ΣDDT, kg	Dieldrin, g
Corn Silage							
Big Otter Creek	3,938	114,020	42.0	0.343	0.004	22.68	265
Big Creek	1,772	49,990	40.5	0.353	0.008	10.50	238
Dedrich Creek	61	1,670	38.5	0.260	0.004	0.27	4
Nanticoke Creek	257	7,030	41.8	0.185	0.007	0.26	29
Total	6,028	172,710				34.21	536
Mean				0.339[1]	0.005		
Hay [2]							
Total	11,792	61,600	10.1			4.10	222
Mean				0.074[3]	0.004		

[1] ΣDDT residue consisted of 21-74 percent DDT, 22-77 percent DDE, and 2-6 percent TDE.
[2] Hay samples were scarcer than silage samples; hence individual watersheds are not listed.
[3] ΣDDT residue on hay by watershed ranged from 0.05 to 0.09 ppm present mainly as actual DDT (75 percent).

TABLE 7. *ΣDDT and dieldrin in milk and beef of four watersheds, southern Ontario—1971*

Watershed	Annual Production (× 1000 kg)	ΣDDT in Extractible Fat,[1] ppm	Composition of DDE and TDE, %	Dieldrin in Extractible Fat, ppm	Residues in Annual Supply, g ΣDDT	Dieldrin
Milk Production [2]						
Big Otter Creek	32,060	0.183	79.1	0.031	234.7	39.8
Big Creek	9,716	0.234	82.9	0.050	90.9	19.4
Dedrich Creek	70	0.230	80.1	0.045	0.6	0.1
Nanticoke Creek	11,716	0.258	86.0	0.057	120.9	26.7
Total	53,562				447.1	86.0
Mean		0.188	81.0	0.035		
Beef Production [3]						
Big Otter Creek	4,182	0.340	82.4	0.042	355.5	43.9
Big Creek	1,468	0.325	81.5	0.051	119.3	18.7
Dedrich Creek	57	0.298	80.9	0.030	4.2	0.4
Nanticoke Creek	275	0.267	85.0	0.052	18.4	3.6
Total	5,982				497.4	66.6
Mean		0.333	82.3	0.045		

[1] Average butterfat content: 4%; average beef content: 25%.
[2] Reference Records, Ontario Milk Marketing Board, Toronto. (Refer to Literature Cited, reference 16.)
[3] Annual Livestock Market Review, 1971, Canada Department of Agriculture. (Refer to Literature Cited, reference 17.)

HAY

Hay samples of limited quantity were obtained from fields in the four watersheds but not from tobacco farms. Residues of ΣDDT varied from 0.05 to 0.09 ppm with a mean composition of 75 percent actual DDT. This suggested that the deposition of DDT was recent, possibly resulting from spray drift. Total hay production in the four watersheds was 61.6 x 10^6 kg in 1971; this contained 4.1 kg ΣDDT and 0.22 kg dieldrin (Table 6).

MILK

Milk produced in the four watersheds totalled 53.6 x 10^6 kg a year (Table 7). The weighted mean residue of ΣDDT in the butterfat from the four watersheds was 0.188 ppm. Where dairy and tobacco production occurred close together, residues ranged from 0.183 to 0.258 ppm. On three farms that raised tobacco and maintained sizable dairy herds, ΣDDT residues in butterfat were 0.39 ppm; average butterfat in the milk was 4.1 percent. These residues were considerably higher than the provincial average of 0.134 ppm reported by Frank et al. (*10*). Composition of ΣDDT in the general milk supply ranged from 79 to 86 percent DDE and TDE.

All milk except that from Big Otter Creek watershed had residues of dieldrin above the 1967-69 provincial average of 0.031 ppm (*10*). A total of 447 g ΣDDT and 86 g dieldrin were present in the annual milk supply of the four watersheds (Table 7). The 447 g DDT present in milk represented only 1.2 percent of the DDT found in hay (4.1 kg) and corn silage (34.1 kg); the 86 g dieldrin in the milk represented 11 percent of that in hay (222 g) and corn silage (536 g).

BEEF

Almost 6 million kg meat a year are produced in the four watersheds (Table 7). Concentrations of ΣDDT ranged from 0.267 to 0.340 ppm with a weighted mean of 0.333 ppm. Over 80 percent of the ΣDDT was present as the two metabolites DDE and TDE. Beef contained a mean of 25 percent fat; the calculated amount of ΣDDT in the meat supply was about 500 g, a quantity slightly greater than that found in the annual milk supply, but representing only 1.3 percent of that present in the corn silage (34.1 kg) and hay (4.1 kg) produced in the area.

Dieldrin residues in beef fat ranged from 0.030 to 0.052 ppm with a weighted mean of 0.045 ppm. Total dieldrin present in the annual production of beef was less than 70 g, slightly lower than the amount found in the milk supply; this represented about 9.3 percent of the dieldrin present in corn silage (536 g) and hay (222 g) (Table 7).

LIVESTOCK WATER SUPPLIES

Water from shallow wells and from spring- and surface-fed farm ponds was collected to determine contamination of livestock water supplies. Of 14 wells, 3 had waters containing residues of 4, 40, and 50 ppt ΣDDT, respectively, but none contained dieldrin. In 11 wells neither ΣDDT nor dieldrin could be detected.

Six spring-fed and nine surface-fed farm ponds were sampled (Table 8); ΣDDT was detected in two spring-fed ponds at 1 and 50 ppt, and in four surface-fed ponds at 9, 35, 60, and 80 ppt. Dieldrin was found in only two surface-fed ponds at 5 and 8 ppt.

These data give mean residues of 6.7 ppt ΣDDT in 14 wells, 8.5 ppt ΣDDT in 6 spring-fed ponds, and 20 ppt ΣDDT and 1.4 ppt dieldrin in 9 surface-fed ponds.

AIR

On 35 days (24-hr periods) between May 1 and August 31, 1971, air was sampled for DDT. Residues occurred on 19 of the 35 days (Table 9). Highest mean concentrations occurred in May (74 ng/m^3); lowest

concentrations occurred in June (2.3 ng/m³). On 10 of the 19 days only *o,p'*- and *p,p'*-DDT were detected; on 2 other days when *o,p'*- and *p,p'*-DDT were high, TDE was also detected. When found, TDE represented 4.3 percent of the ΣDDT, a normal percent component in the formulated product. Hence these residues were considered to have been the result of spray drift. Spraying operations appeared to have occurred on 12 days: 2 in May, 5 in June, 4 in July, and 1 in August. Mean concentration in air on these spray-days was 46.7 ng/m³ air. On 9 of the 12 days, the concentration in air was less than 10 ng/m³, but on the other 3 days ΣDDT concentrations were 15, 48, and 469 ng/m³.

For 7 days, DDE and TDE were present at 21 percent and 14 percent, respectively, of the ΣDDT (Table 9). The presence of these metabolites suggested that residues were derived from either airborne dust particles or volatiles. The fact that the ratio of DDE + TDE/DDT was 1:2 and fairly constant for the 7 days suggested that these residues were derived from airborne particles rather than from volatiles. Mean concentration on these 7 days was 34.5 ng/m³ ΣDDT. Mean residue in air for the 35 days was 27.4 ng/m.³ No dieldrin was detected on any sampling days.

Snow samples were collected in late winter from three main areas in the Big Creek watershed in order to measure DDT fallout. Samples were divided into upper and lower levels to represent early-winter and late-winter snowfalls. Water from the early snowfalls contained an average of 43 ppt ΣDDT with a high of 205 ppt. Late-winter snow contained only 4 ppt (Table 10). Dieldrin was also highest in early-winter snow with a mean of 10 ppt as opposed to 0.7 ppt in late-winter snow. Composition of the ΣDDT suggested that these residues were derived from airborne particles and not volatiles.

It is assumed that residues detected in air and snow might explain the movement of DDT, its metabolites, and dieldrin from the tobacco crop to other crops such as hay and silage that are not sprayed and often are not grown on soil in which tobacco has been produced.

CREEK AND BAY WATER

Big Otter, Big, Dedrich, and Nanticoke Creeks discharged 165, 143, 12.9, and 33.4 million m³ water, respectively, into Lake Erie during 1971 (*11*). Monitoring of waters for organochlorine insecticides was extended on Big Creek from February to October, a period that accounted for 81 percent of the discharge. On the other three creeks, monitoring extended from March to October and accounted for 60 to 69 percent of the discharge (Table 11).

TABLE 8. *Residues of ΣDDT and dieldrin in surface-fed and spring-fed ponds and farm wells, southern Ontario—1971*

Source of Water	No. Sources Sampled	No. Residues Detected ΣDDT	No. Residues Detected Dieldrin	Residues Detected, ppt ΣDDT	Residues Detected, ppt Dieldrin
Surface-fed farm ponds	9	5	7	9, 35, 60, 80	5, 8
Spring-fed farm ponds	6	4	6	1, 50	ND
Farm wells	14	11	4	4, 40, 50	ND

NOTE: ND = not detectable.

TABLE 9. *DDT and its metabolites in air from Big Creek watershed, southern Ontario—1971*

Month of Sampling	No. Days in Operation	No. Days Residues Detected	ΣDDT Mean Residue in Air, ng/m³	DDT Composition, % DDE	TDE	DDT
May	9	4	73.9	5.2	6.0	88.8
June	12	4	2.32	0	0	100
July	5	5	23.8	4.0	2.0	94.0
August	9	6	8.11	9.4	13.6	77.0
May-August	35	19	27.4	5.2	6.1	88.7
Suspected sprayings		12	46.7	0	4.3	93.7
Suspected soil or dust		7	34.5	20.8	14.2	65.0

TABLE 10. *ΣDDT and dieldrin in snow from Big Creek watershed, southern Ontario—1971*

Snow Layer	No. Samples	No. Days Residues Detected	Mean Residue in Water, ppt [1] ΣDDT	Dieldrin	ΣDDT Composition, % DDE	TDE	DDT
Upper level	6	4	4	0.7	12	0	68
Lower level	6	4	43	10.0	14	10	76

[1] Ppt = parts per trillion.

TABLE 11. *Concentration and amount of ΣDDT and dieldrin in creeks, southern Ontario—1971*

Month	Waterflow [1] ($\times 10^6 m^3$)	Concentrations in Water, ppt [2] ΣDDT	Dieldrin	Composition, % DDE	TDE	DDT	Residues, g ΣDDT	Dieldrin
Big Otter Creek								
March	28,659	15.2	1.8	18	48	34	436.0	51.6
April	31,406	18.0	3.0	22	33	45	565.0	94.2
May	14,240	21.4	0.4	13	35	52	305.0	5.70
June	7,214	14.6	0.9	46	14	40	105.0	6.49
July	8,169	9.7	0.8	32	48	40	79.2	6.54
August	4,910	5.7	0.1	32	28	40	28.0	0.49
September	5,486	4.8	0.0	30	50	20	26.3	0.00
October	7,901	4.7	0.1	19	60	21	37.1	0.79
Mar.-Oct.	107,985	14.6	1.5				1,581.6	165.31
% Total	65.4						76.9	87.9
Jan., Feb., Nov., Dec.	57,118	4.7	0.1				268.0	5.71
Annual total	165,103						1,849.6	171.52
Annual mean		11.2	1.0					
Big Creek								
February	13,628	5.5	0.2	17	61	21	75.0	2.73
March	37,408	30.0	0.8	18	50	32	1,122.0	29.9
April	24,062	43.0	3.0	15	50	35	1,035.0	72.2
May	11,383	16.8	1.0	17	26	57	191.0	11.4
June	7,776	11.8	0.2	51	17	32	91.8	1.56
July	4,910	8.8	1.5	30	28	42	43.2	7.37
August	4,910	6.9	0.1	26	29	45	33.9	0.49
September	5,875	11.0	0.1	16	29	55	64.6	0.59
October	6,250	7.1	0.1	17	45	38	44.4	0.63
Feb.-Oct.	116,202	23.2	1.1				2,700.9	126.87
% Total	81.1						94.0	96.9
Jan., Nov., Dec.	27,100	6.3	0.1				171.0	2.71
Annual total	143,302						2,871.9	129.58
Annual mean		20.0	0.9					
Dedrich Creek								
March	554	23.1	2.5	15	58	27	12.3	1.39
April	3,339	20.0	3.0	15	50	35	66.8	10.0
May	1,629	18.1	0.4	14	41	45	29.5	0.65
June	890	45.1	0.4	9	46	45	40.1	0.36
July	281	8.1	0.8	28	36	36	2.28	0.22
August	250	8.1	0.4	28	36	36	2.03	0.10
September	337	7.4	0.3	27	41	32	2.49	0.10
October	379	6.5	0.2	25	47	28	2.46	0.03
Mar.-Oct.	7,659	20.7	1.7				158.46	12.90
% Total	59.5						82.4	92.5
Jan., Feb., Nov., Dec.	5,215	6.5	0.2				33.9	1.04
Annual total	12,874						192.36	13.94
Annual mean		14.9	1.1					
Nanticoke Creek								
March	12,812	11.1	1.2	15	57	28	142.2	15.37
April	4,882	16.0	2.0	25	48	37	78.1	9.76
May	1,821	23.9	1.3	18	31	51	43.5	2.37
June	2,497	18.9	2.5	34	15	51	47.2	6.24
July	241	11.2	1.5	25	35	40	2.70	0.36
August	76	5.5	0.1	25	35	40	0.42	0.01
September	242	7.0	1.0	14	29	57	1.69	0.24
October	455	6.2	0.5	15	48	37	2.32	0.23
Mar.-Oct.	23,026	13.8	1.5				318.63	34.53
% Total	68.9						77.9	80.6
Jan., Feb., Nov., Dec.	10,371	6.2	0.5				64.3	5.19
Annual total	33,397						382.93	39.77
Annual mean		11.5	1.2					
Four Watersheds								
Annual total	354,676						5,296.8	354.81
Annual mean		14.8	1.0					
Inner Long Point Bay								
Mouths of Big and Dedrich Creeks	89,106 [3]	12.5	0.3	45	22	33	409.9	8.91
Middle of bay (3 miles)		1.3	ND	100	0	0		
Edge of Inner and Outer Long Point Bay		Trace	ND	—	—	—		

[1] Water Flow Data, Environment Canada. (Refer to Literature Cited, reference 11.)
[2] Ppt = parts per trillion.
[3] Berst and McCrimmon. (Refer to Literature Cited, reference 12.)

The highest concentration of ΣDDT in the waters of these four creeks occurred during the spring months, March to May, when discharge volumes were highest. Concentrations rose to 21, 43, 45, and 24 ppt, respectively, in Big Otter, Big, Dedrich, and Nanticoke Creeks coincident with spring thaw and runoff, cultivation of land, and spraying of the rye cover crop for cutworm control. Concentrations in water declined in midsummer to 5-8 ppt in spite of rainfall that was heavier in the period from July to September (Table 12). ΣDDT in water in Long Point Bay just off the creek mouths contained lower concentrations (12.5 ppt) than either the waters from Big Creek (20 ppt) or Dedrich Creek (14.9 ppt), indicating mixing and dilution as water enters the bay.

Three miles off the mouths of these creeks in the middle of the Inner Bay, residues had declined to 1.3 ppt ΣDDT and were all present as DDE (Table 11). Water collected at the juncture of Inner and Outer Bays contained only traces of DDT. It is estimated that Inner Bay contained 89,106 thousand m^3 water (*12*) which could contain 410 g ΣDDT and 8.9 g dieldrin.

During the months of February, March, and April, the largest fraction of ΣDDT was present as TDE (Table 11, Fig. 2). The parent compound DDT was lowest in February and increased to become greater than TDE in May; the DDE fraction remained small and unchanged. In the period of May to September, DDT comprised the greatest fraction as it rose to a peak; it then declined in October. The TDE fraction declined to its lowest level in June, but increased again in July. The DDE fraction almost doubled during June to August to 30 percent. By October TDE again became the predominant fraction; DDE and DDT declined and remained in roughly equal proportions. Increase in DDT coincided with its use on rye and the cultivation of the soil for planting the tobacco crop.

The largest resident load of ΣDDT discharged by the four creeks occurred between March and April when the water discharge volumes were at their highest; this was over 1000 g ΣDDT in the case of Big Creek during March and April (Fig. 3). Big Otter Creek, which discharged a greater volume of water than Big Creek, delivered only half the quantity of DDT to Lake Erie during these peak months. Dedrich, a small creek, discharged only 67 g in April.

Site 6, the main sampling site on Nanticoke Creek, was located in the tobacco belt; the flow meter was downstream. Water at site 6, which was sampled regularly, contained a peak of 24 ppt in May and a low of 5.5 ppt in August. The six water samples collected at the flow meter near the town of Nanticoke had a peak of 35 ppt in May and 4 ppt in August. It was not clear where the additional DDT between the two sites originated because the volume of water almost trebled. Because the residue data at site 6 were more complete than at the flow meter, these site data were used to estimate residue loads. Nanticoke Creek appeared to carry its largest load of DDT, 142 g, in March.

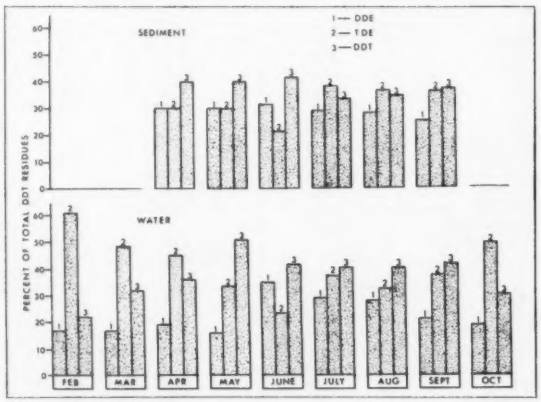

FIGURE 2. *Monthly composition of ΣDDT in water and sediment of creeks in study area, 1971*

TABLE 12. *Frequency and amount of rainfall, 1971* [1]

Month	Frequency of Rainfall, Days — Range, cm				Rainfall, cm [2]
	0.01-1.00	1.01-2.00	2.01-3.00	Over 3.01	
January	10	0	0	0	4.72
February	10	3	0	0	8.26
March	3	2	0	0	3.68
April	5	2	0	0	3.73
May	8	0	0	1	4.06
June	8	2	0	0	4.27
July	6	1	1	0	5.36
August	6	0	2	1	9.68
September	10	0	1	0	4.55
October	6	1	0	0	3.86
November	9	2	0	0	5.00
December	9	5	0	0	11.63
Total	90	18	4	2	68.80

[1] Climatological Station Report, Canada Department of Agriculture, Delhi, Ontario, 1971. (Refer to Literature Cited, reference 18.)
[2] Of total rainfall, snow comprised 3.05 cm in January, 3.56 cm in February, 2.03 cm in March, 1.27 cm in April, 1.14 cm in November, and 2.06 cm in December.

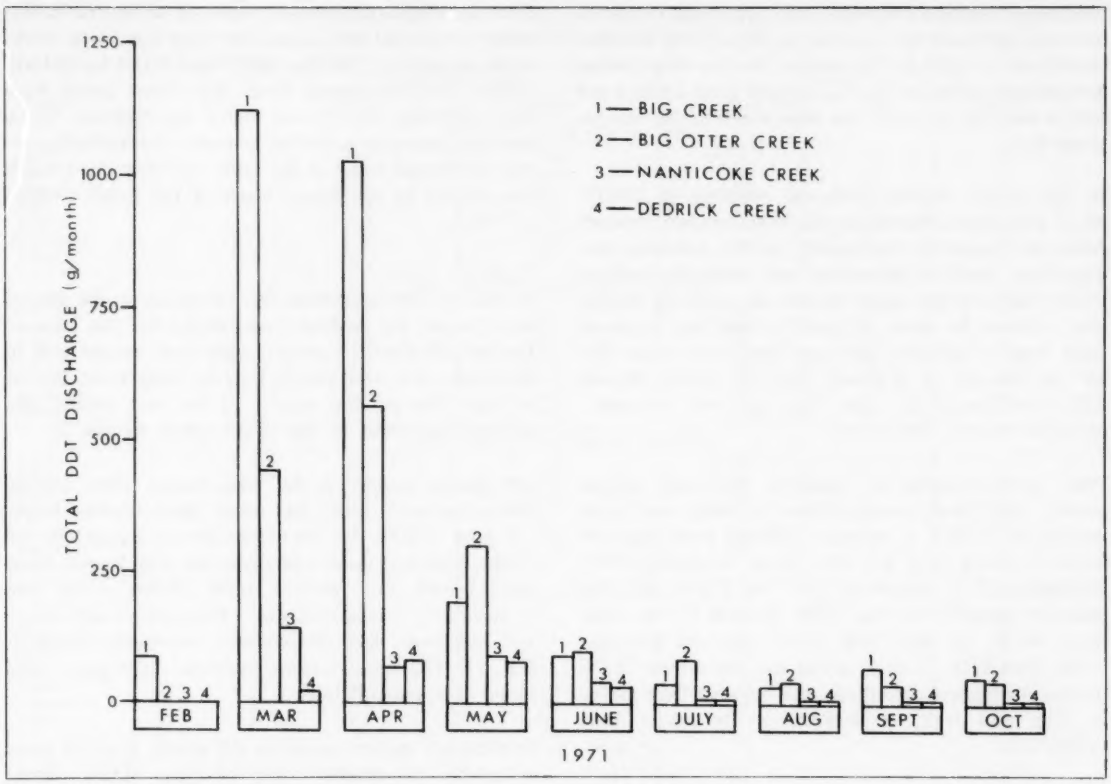

FIGURE 3. *ΣDDT residues in discharge water entering Lake Erie from the four creeks in the study area, 1971*
(NOTE: no data available on creeks 2, 3, or 4 for February)

Because the concentration of ΣDDT in creek water was highest in the spring and lowest in the fall, an estimate based on lower concentrations was made for quantities of DDT and dieldrin through late fall and early winter. Big Creek carried the greatest annual residue load of ΣDDT which was estimated at 2,872 g. Big Otter Creek carried the second-highest load, 1,850 g, and Nanticoke and Dedrich Creeks carried 383 and 192 g, respectively. Collectively, all four watersheds carried a total of 5,297 g/year, an average of 14.5 g/day, into Lake Erie. This represented that portion of DDT and its metabolites that was either dissolved in the water or on particles that passed through Whatman No. 1 filter paper and not that transported on removable suspended sediment.

Concentrations of dieldrin in creek water were also highest in March and April, rising to a level of 2 to 3 ppt. Concentrations declined during the summer and fall to between one-fifth and one-tenth these levels. Dieldrin concentrations represented between one-tenth and one-hundredth the level of ΣDDT. Residues of dieldrin entering Lake Erie were also highest in March and April; the greatest quantity, 94 g, was carried in the peak month of April by Big Otter Creek. The largest quantity carried by Big Creek, 72 g, occurred in April. Nanticoke and Dedrich Creeks carried peak amounts of 15 and 10 g, respectively, in these same months. The annual quantity reaching Lake Erie was 172 g from Big Otter Creek, 130 g from Big Creek, 40 g from Nanticoke Creek, and 14 g from Dedrich Creek. Hence the annual discharge of the four watersheds was 354.7 g, almost 1 g/day.

CREEK AND BAY SEDIMENTS

Sediments taken at the interface between creekbed and water showed weekly variations in the concentrations of ΣDDT and dieldrin as they shifted downstream (Table 13). Highest mean ΣDDT residues were observed in sediments from Dedrich Creek (141 ppb) but were much lower in Nanticoke Creek (55.6 ppb), Big Otter Creek (41.3 ppb), and the lower reaches of Big Creek (38.7 ppb). Sediments in Dedrich Creek contained almost 200 ppb ΣDDT in June; however, this level declined steadily to 93 ppb by September. Greatest residue levels in Big Otter Creek, 58.6-61.0 ppb, were found in August and September. The highest sediment

residues in Nanticoke Creek, 70-95 ppb, were measured between April and June at site 6; these levels declined steadily to 23 ppb by September. At the flow station downstream, sediment residues ranged from 25 to 1 ppt with a mean of 10 ppb, less than one-fifth the residue at site 6.

In Big Creek, highest sediment residues of ΣDDT, 45-51 ppb, were observed on the lower reaches between June and August. In the middle reaches, sediment residues from April to September were relatively uniform (13-21 ppb); in the upper reaches the sediment residue level reached its peak, 42 ppb, in May and declined until August. Sediment collected from Long Point Bay off the mouths of Big and Dedrich Creeks showed ΣDDT residues of 28.7 ppb; they were only 4.3 ppb 3 miles into the bay (Table 13).

Peak concentrations in sediment occurred several months after peak concentrations in water and composition of ΣDDT in sediment differed from that observed in water (Fig. 2). The parent compound DDT predominated in sediments from Big Creek and was generally greater than the DDE fraction in the other three creeks. In Big Creek, DDE was also generally higher than TDE. In early spring and late summer TDE reached its highest percentage, but between these times, in May and June, it declined to its lowest level (Table 13).

Composition of ΣDDT in sediment from Nanticoke Creek differed from that of Big Creek. In the Nanticoke, TDE predominated in samples collected during 4 of the 6 months; DDT and DDE were in approximately equal quantities. Sediments from Dedrich Creek contained predominately DDT during April, May, and June, and predominately TDE during July, August, and September. In Big Otter Creek the composition of ΣDDT showed no definite pattern (Table 13).

In Long Point Bay, TDE predominated in samples collected near the mouths of Dedrich and Big Creeks, but 3 miles out in the bay, TDE and DDE were present in about equal amounts.

Dieldrin residues in sediments from all four creeks were of a similar level; the highest mean residue, 2.1 ppb, was found in Big Otter Creek, and the lowest, 0.7 ppb, was from Dedrich Creek. Residues in sediment in Long Point Bay close to the outlets of Big and Dedrich Creeks contained 0.6 ppb dieldrin although dieldrin could not be detected 3 miles out in the bay. Dieldrin residues in general appeared in quantities between one-twentieth and one-fortieth the levels of ΣDDT.

In 1971 Environment Canada (*11*) reported that Big Otter Creek carried 54,368,000 kg and Big Creek carried 13,438,000 kg suspended sediments into Lake Erie. Based on the residues found in sediments in this study, movement into Lake Erie from these two creeks could amount to 1.60 kg ΣDDT and 0.114 kg dieldrin (Table 14); movement from Big Creek could have been 0.39 kg ΣDDT and 0.013 kg dieldrin. These amounts represent a similar quantity of insecticide carried by filtered water in Big Otter but only one-seventh that carried by the filtered water of Big Creek (Tables 11,14).

FISH

A total of 289 individual fish belonging to 24 species were caught for analysis from the creeks, the lake, or the bay. Of these 24 species, eight were caught only in the creeks, ten were caught only in Long Point Bay or in Lake Erie off the mouths of the four creeks, and six were common to the two systems (Table 2).

All species caught in the lake, except coho salmon *(Oncorhychus kisutch)*, had mean tissue residues below 1.0 ppm ΣDDT. Of the eight species caught in the creeks, four had mean tissue residues over 1 ppm; these were brown and rainbow trout *(Salmo trutta* and *S. gairdneri)*, largemouth bass *(Micropterus salmoides)*, and blacknose dace *(Rhinichthys atratulus)* (Table 2, Fig. 4). The highest tissue residues, 3.86 ppm, were found in largemouth bass.

Of the eight species caught in the creeks, five had mean extractible fat residues over 15 ppm ΣDDT. These included largemouth bass with the highest level, 51 ppm; brown and rainbow trout; creek chub *(Semotilus atromaculatus);* and central mudminnow *(Umbra limi)*. Residue levels over 15 ppm ΣDDT in the fat were found in two species caught in the lake; these were smallmouth bass *(Micropterus dolomieui)* and coho salmon. Although the species were not common to the two bodies of water, there was a greater concentration of ΣDDT in these species confined to the creeks. These differences were very apparent in species caught in both systems. Of six species common to the two systems, tissue residues of ΣDDT ranged from 4 to 15 times higher in those members caught in the creeks than in those caught in the lake (Fig. 4).

Dieldrin residues were over 0.1 ppm in the tissues of only one species, largemouth bass. Only one species, blacknose dace, had mean dieldrin residues between 0.05 and 0.1 ppm. Both species were caught in the creeks. Of the 10 species caught in the lake, three had undetectable dieldrin residues and many of the remainder had residues at or below 0.01 ppm. When residues in extractible fat are considered, only one species caught in the lake, black crappie *(Pomoxis nigromaculatus)*, had dieldrin residues over 1 ppm. However, seven species caught in the creeks contained residues over 1 ppm and in largemouth bass the residues were almost 5 ppm.

TABLE 13. *Composition and concentration of ΣDDT and dieldrin in sediments of four water courses in four watersheds, southern Ontario—1971*

Month	Dried Sediment, ppb		Composition, %		
	ΣDDT	Dieldrin	DDE	TDE	DDT
Big Creek—Upper Reaches; Sand					
April	19.4	ND	26	36	38
May	41.8	0.8	30	17	53
June	17.6	0.6	39	24	37
July	15.5	0.4	54	16	30
August	10.9	0.6	41	26	33
September	11.6	0.4	29	36	35
Mean	19.5	0.5			
Big Creek—Middle Reaches; Sandy Silt					
April	20.8	2.0	43	31	26
May	19.0	0.7	36	24	40
June	12.8	0.2	33	20	41
July	19.7	0.4	29	26	45
August	15.7	0.4	22	21	57
September	20.0	0.5	20	26	54
Mean	18.0	0.7			
Big Creek—Lower Reaches; Fine Sand					
April	25.4	0.4	20	25	55
May	30.6	2.0	29	16	55
June	51.4	0.5	34	13	53
July	50.2	2.1	33	26	41
August	44.8	0.4	29	34	37
September	29.7	0.4	28	34	38
Mean	38.7	1.0			
Big Otter Creek; Sandy Silt					
April	24.4	3.0	33	43	24
May	34.6	0.3	38	23	29
June	40.5	1.4	53	21	26
July	28.8	1.3	29	36	35
August	58.6	3.6	27	28	45
September	61.0	3.0	25	36	44
Mean	41.3	2.1			
Dedrich Creek; Sandy Silt					
April	162.4	0.1	22	25	53
May	129.6	1.5	24	36	40
June	198.2	0.8	19	38	43
July	138.3	1.3	22	48	30
August	124.4	0.1	20	43	37
September	93.0	0.4	20	45	35
Mean	141.0	0.7			
Nanticoke Creek; Sandy Silt					
April	73.0	2.4	45	25	30
May	94.8	0	30	43	27
June	70.3	1.4	23	34	43
July	45.7	2.4	32	46	32
August	26.6	1.4	35	43	22
September	23.0	3.0	30	39	31
Mean	55.6	1.9			
Long Point Bay					
Mouth: May-September	28.7	0.6	18	72	10
Middle of bay: 3 miles	4.3	ND	46	48	6

NOTE: ND = not detected.

TABLE 14. *ΣDDT and dieldrin carried on suspended solids by Big Creek and Big Otter Creek to Lake Erie—1971*

Creek	Suspended Solids, kg/yr	Average Concentration, ppb		Amount Carried to Lake Erie, kg	
		ΣDDT	Dieldrin	ΣDDT	Dieldrin
Big Otter Creek	54,368,000	41.3	2.1	1.60	0.114
Big Creek	13,438,000	38.7	1.0	0.393	0.013
Total	67,806,000			1.993	0.127

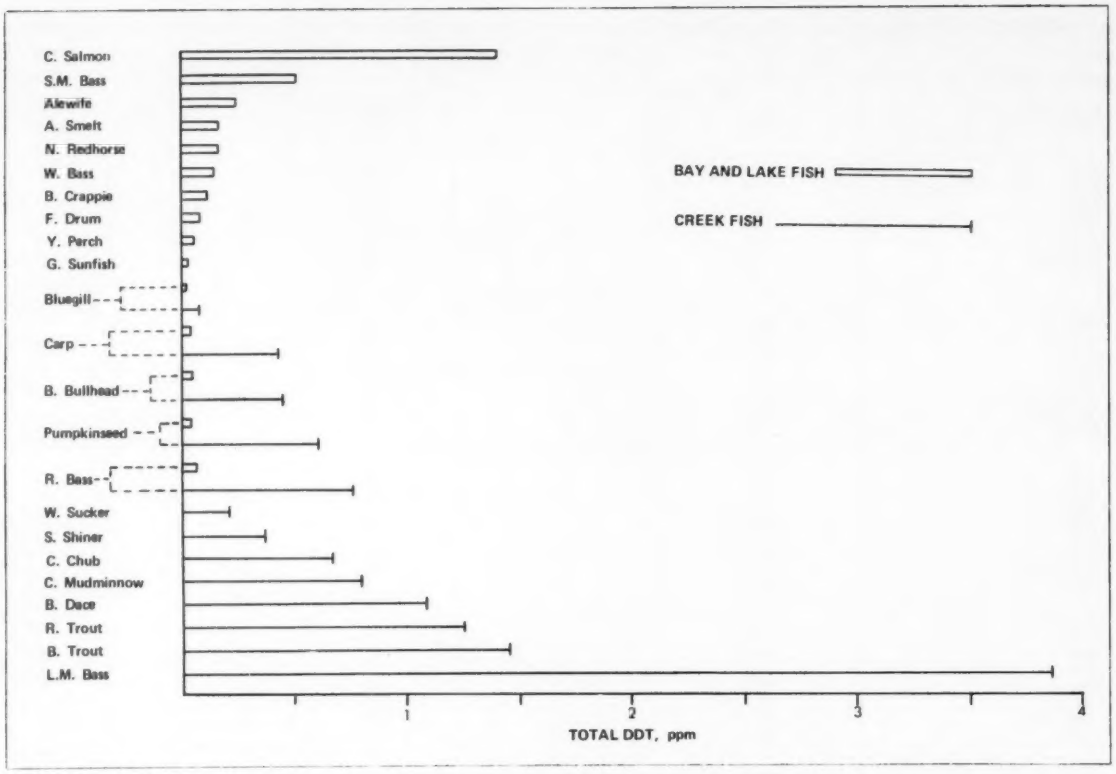

FIGURE 4. *ΣDDT concentrations in tissues of 23 fish species caught in creek and lake environments, southern Ontario—1971*

Creek Fish

Among the species caught in the creeks, blacknose dace and spottail shiners *(Notropis hudsonius)* were members of the lowest trophic levels. Yet their tissue and fat residues of both ΣDDT and dieldrin were comparatively high. Bottom-feeding white suckers *(Catostomus commersoni)*, which have lower fat content in the tissues, had lower residues of DDT and dieldrin than those in dace and shiner; but based on extractible fat their residues were higher. A marked increase in ΣDDT was observed in both extractible fat and total body burdens as the average weight of white suckers increased. Creek chub, an important link in the aquatic food chain, contained almost 0.7 ppm ΣDDT in the tissue and 16 ppm in the extractible fat. Dieldrin levels were 0.04 ppm in the tissue and 1.17 ppm in the fat.

Piscivores caught in the creek included largemouth bass, and brown and rainbow trout. Largemouth bass accumulated the highest residues of ΣDDT and dieldrin in tissue and extractible fat. As the size of the rainbow trout increased, tissue and fat concentrations showed little change although the body load increased rapidly. In fish weighing an average of 6,400 g body load, 8.6 mg ΣDDT and 0.45 mg dieldrin were accumulated.

Body burdens in the creek-caught species increased from 0.07 μg ΣDDT in tiny white suckers weighing an average of 6 g to 8,640 μg in the largest rainbow trout. This represented an accumulation of 1.2×10^5. With dieldrin, the observed increase was from 0.02 μg in tiny white suckers (Table 2) to 448 μg in large rainbow trout, an accumulation of 6.4×10^3 (Table 15).

Lake Fish

Plankton feeders caught in the lake had ΣDDT residues up to 0.25 ppm in the tissue but only trace quantities of dieldrin. The bottom-feeding redhorse *(Moxostoma macrolepidotum)* contained low residues similar to those of the plankton feeders. Black crappies feeding on crustaceans and small fish showed only slightly elevated residues. Among the piscivores, smallmouth bass contained an elevated tissue and fat concentration of ΣDDT and dieldrin and exhibited a buildup in body loads with increasing fish size. Coho salmon contained the highest residue of ΣDDT.

The range from the lowest body load of 2 μg ΣDDT in American smelt *(Osmerus mordax)* to the highest body load of 6,652 μg in coho salmon represents an accumulation of 3.3×10^3. With dieldrin the range was from a nondetectable level in green sunfish *(Lepomis cyanellus)* to 11.6 μg in smallmouth bass.

TABLE 15. *Biomagnification of ΣDDT and dieldrin in the aquatic environment of watershed creeks and Lake Erie, southern Ontario—1971*

Insecticide	Material	Residues in 1 g	Magnification
	Watershed Creeks		
ΣDDT	Water	1.5×10^{-11}	
	Sediment	4.1×10^{-8}	2.7×10^{3}
	White sucker (smallest)	1.2×10^{-8}	8.0×10^{2}
	Rainbow trout (largest)	1.4×10^{-6}	9.3×10^{4}
	Largemouth bass (highest residues)	4.8×10^{-6}	3.2×10^{5}
Dieldrin	Water	1.0×10^{-12}	
	Sediment	1.9×10^{-11}	1.9×10^{3}
	White sucker (smallest)	3.3×10^{-9}	3.3×10^{3}
	Rainbow trout (largest)	7.0×10^{-8}	7.0×10^{4}
	Largemouth bass (highest residues)	2.3×10^{-7}	2.3×10^{5}
	Bay and Lake Erie		
ΣDDT	Water	6.9×10^{-12}	
	Sediment	1.7×10^{-8}	2.5×10^{3}
	Bluegill (lowest residues)	3.1×10^{-8}	4.5×10^{3}
	Coho salmon (largest)	2.3×10^{-6}	3.3×10^{5}
Dieldrin	Water	1.5×10^{-13}	
	Sediment	3.0×10^{-10}	2.0×10^{3}
	White bass (large)	1.7×10^{-8}	1.1×10^{5}

Creek and Lake Fish

Among the fish caught in both the creeks and the bay, the plankton-feeders, rock bass *(Ambloplites rupestris)* and pumpkinseed *(Lepomis gibbosus)*, had residues 10 to 15 times higher in those members caught in the creek than in those caught in the bay or lake (Fig. 4,5). The bottom-feeders, brown bullhead *(Ictalurus nebulosus)* and carp *(Cyprinus carpio)*, contained residues of similar differences in magnitude. The marsh-feeding bowfin *(Amia calya)* had low residues of both DDT and dieldrin. No true piscivore was caught in both the creek and lake waters for comparison.

Discussion

From the data collected it is possible to prepare an inventory of ΣDDT present in the four watersheds and the amount being slowly removed in agricultural produce and by natural processes. The soil that acts as a reservoir was estimated to contain 324,840 kg ΣDDT and 14,515 kg dieldrin in 1971.

Over the past 11 years (1961-71) it was estimated that 882,730 kg DDT was added to the soil. From these data it was proposed that the half-life was between 3 and 4 years. Data compiled by Edwards showed that at

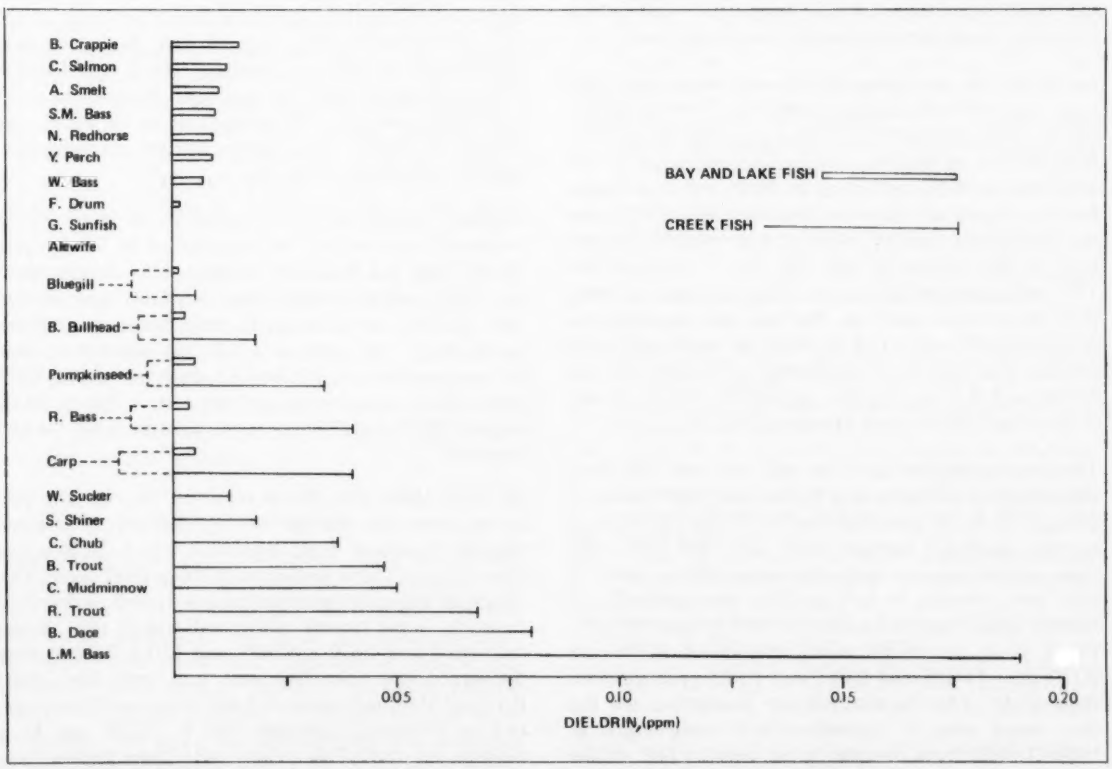

FIGURE 5. *Dieldrin concentrations in tissues of 23 fish species caught in creek and lake environments, southern Ontario—1971*

dosages between 1.11 and 2.80 kg/ha., disappearance of 95 percent occurred in 4 to 30 years depending on soil, weather, and location (*13*). In the present study 95 percent disappearance was predicted to occur in 15 to 18 years, well within the span reported by Edwards. The average percent of parent DDT reported by Edwards that remained after 3 years was about 50 percent; however, his range was from 26 to 78 percent. In this study the figure was close to 80 percent.

Mean residues of ΣDDT found in soil agreed closely with the findings of Harris and Sans, in which the averages of four farms taken in 1964, 1966, and 1969 amounted to 3.06, 4.56, and 3.38 ppm ΣDDT, respectively (*5*). In the present survey, soils recently cropped with tobacco contained mean residues between 3.88 and 4.36 ppm for each of the four watersheds. At the end of a 2- to 3-year rotation the same soils had mean residues ranging from 2.33 to 3.44 ppm. Composition of DDT at the beginning and end of the tobacco/grain rotation showed little change, making it difficult to attribute disappearance to degradation. In woodlots, considerable degradation of DDT to DDE was evident (Table 3) compared to rye and tobacco fields.

Air did not appear to account for volatile losses from the soil. Instead, some samples indicated that most of the DDT was derived from spray drift and others indicated that particulate matter was transported by air.

In the annual marketing of tobacco, milk, and meat from the four watersheds, a total of 53.5 kg ΣDDT and 2.13 kg dieldrin were removed. This represented 0.18 percent of the pesticide applied, assuming it was all from the 1971 application of DDT, which is doubtful. It is significant, however, that both the ΣDDT and the dieldrin removed by tobacco represented 0.035 percent of that resident in the soil used to produce the 1971 crop. Residues in hay and silage appeared to come from either spray drift or the soil and amounted to 38.3 kg ΣDDT and 0.76 kg dieldrin. Amounts found in meat and milk were calculated to be only 0.95 kg ΣDDT and 0.16 kg dieldrin, a fraction of that found in the silage and hay used as animal feed.

The average residue level in milk fat from the four watersheds, 0.188 ppm, was higher than the Provincial average of 0.134 ppm reported by Frank et al. from surveys conducted between 1967 and 1969 (*10*). The same authors have recently determined residue levels in milk from counties in the Lake Erie watershed (*1*). In surveys which they conducted in 1968-69 and 1970-71, Frank et al. discovered mean residues of 0.186 and 0.122 ppm ΣDDT and 0.041 and 0.035 ppm dieldrin, respectively (*14*). Because tobacco production was the only major area of agriculture still using DDT in 1970-71, milk from counties in the tobacco belt, unlike milk from other counties in the Lake Erie watershed, did not show a decline in ΣDDT residues.

Total annual removal of ΣDDT and dieldrin dissolved in water was estimated at 5,297 g ΣDDT and 354.8 g dieldrin. Data collected by the 1971 Water Survey of Canada on Big Otter and Big Creeks determined that the amount of suspended sediment carried into Lake Erie by these two creeks was 54.4 and 13.4 x 10^6 kg/year (*11*). Bed sediments from Big Otter Creek averaged 41.3 ppb ΣDDT and 2.1 ppb dieldrin; those from the lower reaches of Big Creek averaged 38.7 ppb ΣDDT and 1.0 ppb dieldrin. If it can be assumed that suspended material contained residues similar to those of bed sediments, then it can be estimated that 1,600 g ΣDDT and 114 g dieldrin could have been discharged from Big Otter Creek and 393 g ΣDDT and 13 g dieldrin could have been discharged by Big Creek into Lake Erie on suspended material. In other studies finer sediments tended to contain residues of ΣDDT and dieldrin higher than those of coarser sediments (*7*); therefore, it might be reasonable to predict that suspended material may carry higher and not lower residues than bed sediments.

Because no data were available on the movement of suspended solids from Nanticoke and Dedrich Creeks, no estimate of sediment residues can be made. Figures from the present study were only slightly higher than those of Miles and Harris (*6*), showing close agreement. In the case of Nanticoke Creek, sediment at the town of Nanticoke was only 10 ppb; hence the load carried by this creek is probably small. On Dedrich Creek residues in sediment were high. Because the creek is sluggish and the discharge volume is small, the amount of ΣDDT and dieldrin carried on suspended material is probably small also.

Residues leaving the four watersheds in water or on suspended material can be estimated to be 7.3 kg/year ΣDDT and 0.5 kg/year dieldrin. This represents a loss of 38 and 2.6 mg/ha./year for DDT and dieldrin on a total watershed basis. If these losses are confined to the 40,455 ha. tobacco soil in the watersheds, then the amounts lost are 180 and 12 mg/ha./year for DDT and dieldrin, respectively, and represent a loss of 0.002 percent DDT and 0.003 percent dieldrin from the soil reservoir.

In 1971 Miles and Harris reported that for the preceding year the average weekly delivery of organochlorine insecticide from Big Creek into Lake Erie had been 50 g (*6*). This represents 2,600 g DDT/year. This discharge included suspended matter as well as dissolved pesticide. In the present study conducted in 1971, it was calculated that 2,872 g ΣDDT and 130 g dieldrin were discharged into Lake Erie each year from Big Creek. Riverbed shift and suspended sediments could have carried an additional estimated 393 g ΣDDT and 13 g dieldrin into Lake Erie (Table 14). These figures from the present study are only slightly higher than those from Miles and Harris (*6*), showing close agreement.

The mean residues in water from Big and Dedrich Creeks were 20.0 and 14.9 ppt ΣDDT and 0.9 and 1.1 ppt dieldrin. In Long Point Bay close to the outlets of these two creeks, residues in water were 12.5 ppt ΣDDT and 0.3 ppt dieldrin. Three miles off the mouth in Long Point Bay ΣDDT residues were 1.3 ppt and dieldrin could not be detected. At the edge of Inner Long Point and Outer Long Point Bays, residues of ΣDDT were down to a trace. A marked dilution of residues occurred in water passing from creek to bay to lake.

Mean residues in sediment were 38.7 and 141 ppb ΣDDT and 1.0 and 0.7 ppb dieldrin from the lower reaches of Big Creek and from Dedrich Creek. Close to shore in Long Point Bay the mean residues were 28.7 ppt ΣDDT and 0.6 ppt dieldrin; in the middle of Long Point Bay sediments contained only 4.3 ppb ΣDDT and no dieldrin. These data also illustrate dilution.

Fish species caught in the creeks had markedly higher residues than those caught in the lake. Tissue levels in 10 species caught in the lake ranged from 0.043 to 2.38 ppm ΣDDT compared to tissue levels of 0.01 to 3.86 ppm ΣDDT in eight species caught in the creeks. Tissue levels of dieldrin ranged from nondetectable to 0.024 ppm in 10 species from the lake and 0.004 to 0.190 ppm for eight species from the creeks. These data emphasized differential accumulation of ΣDDT and dieldrin according to contamination of the location and the species involved. It is significant that of the six species caught in both systems, those members caught in the creeks had much higher residues than those caught in the lake. In both creek and lake, magnifications of ΣDDT and dieldrin from water to the highest concentrations in fish tissue were of the order of 10^5.

The ΣDDT and dieldrin carried to Lake Erie in the four watersheds represented only 0.003 percent and 0.004 percent of those resident in the soil in 1971. The presence of this amount in the aquatic environment was reflected by the higher residues in fish caught in the creek than in the lake. However, the level of ΣDDT in fish tissue remained below the 5 ppm tolerance permitted in commercial fish.

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Persistence and Movement of BHC in a Watershed, Mount Mitchell State Park, North Carolina—1967-72 [1]

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ABSTRACT

An experimental area in Mount Mitchell State Park, North Carolina, was sprayed with BHC, benzene hexachloride, at an average rate of 11.2 kg/ha. to control the balsam woolly aphid (Adelges piceae). *Residues were 31 and 585 ppm in soil and litter, respectively, 1 month after spraying. A high percentage of the residue appeared to remain in the treated area; contamination of streams draining the area was minimal. BHC present in surface litter after application slowly moved into surface soil. The residue level in surface soil reached a high of 58 ppm 1.5 years after application. At this time the concentration in litter averaged 134 ppm. Residues in soil and litter were 32 and 27 ppm, respectively, 5 years after application. Concentrations of BHC in animals were unrelated to trapping locations.*

Introduction

In North Carolina infestations of balsam woolly aphid (*Adelges piceae*) were first observed in 1957 among stands of Fraser fir (*Abies fraseri*) in the western North Carolina mountains (*1*). Since that time stands have been severely reduced in many areas; in some areas all mature trees have been killed. Seedlings developing in such areas are also attacked and succumb within 1 to 7 years (*2*).

Except for commercial plantings for Christmas tree production, the value of Fraser fir is primarily, if not entirely, aesthetic. The tree occurs naturally in North Carolina only at altitudes above 1200 m.

Both lime sulfur (calcium polysulfides) and BHC (1,2,3,4,5,6-Hexachlorocyclohexane) have been used to control the balsam woolly aphid. Because lime sulfur has no residual activity, retreatment for several years is required for effective control. Therefore, control with lime sulfur is more expensive than it is with BHC. However, the potential for accumulation in nontarget biota is greater with BHC.

Several investigators have studied the depletion of BHC and lindane, the gamma isomer of BHC, from soil. Using electron-capture/gas-liquid chromatography (EC/GLC), Nash and Woolson (*3*) found an average residue of 10 percent from two BHC applications of 56 and 224 kg/ha. 14 years later. Lichtenstein and Polivka (*4*) recovered an average of 41 percent by a colorimetric method 11 years after application of 0.3, 2.8, 5.6, and 11 kg/ha. BHC. In another study Lichtenstein et al. (*5*) detected 0.2 percent lindane, originally applied at rates of 11 and 112 kg/ha., 15 years later.

Although BHC and lindane are less persistent than most other chlorinated hydrocarbon insecticides (*3,5*), residues remained for several years in soils. Longevity was influenced by soil type, moisture level in soil, evaporation of water from soil, temperature, and other factors (*6,7,8*). Soil microorganisms appear to be involved in decomposition of lindane (*9*) and other isomers of BHC (*10*) in submerged soils. The residual properties of BHC which contribute to its long-term control of the balsam woolly aphid may be undesirable from the standpoint of nontarget biota.

The investigation reported here was undertaken to study the persistence of BHC in a typical Fraser fir stand. In

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conjunction with a spray operation on the north side of the Mount Mitchell peak in 1967, experimental plots and sampling schemes were established for several reasons: to study the deposition and persistence of BHC in surface litter and soil within the sprayed area; to determine residues in water and sediment of small streams within and below the treated area; and to measure residues in small mammals in sprayed and unsprayed areas.

Experimental Procedures

Three 0.04-ha. plots were marked in Mount Mitchell State Park, North Carolina, on the north side of the peak at an elevation of about 1900 m. Plot I contained an intermediate stand of Fraser fir; plot II, a relatively young stand of Fraser fir; and plot III, an old stand of Fraser fir.

BHC was applied to a 13-ha. area on the north side of the mountain June 28—August 30, 1967. Each tree was sprayed individually with a 1:100 dilution of BHC concentrate containing 11.1 percent of the gamma isomer and 16.7 percent of other isomers. The 13-ha. area was sprayed at an average rate of approximately 11.2 kg/ha. BHC. The three experimental plots lay within the sprayed area. Plot III was sprayed June 28, 1967; plots I and II were sprayed July 5 the same year.

Surface litter was defined as the organic material above, but not including, the 02 horizon (*11*). A sample consisted of litter from 20 random sites (grab subsamples) within each experimental plot. Each plot was sampled twice on each sampling date. Researchers randomly took two 20-core samples of soil, each core 2.5 cm in diameter and 15 cm deep, from all three plots several times during a 5-year period after application.

Because the area treated in 1967 had been sprayed with BHC, control samples of soil and litter were taken from an unsprayed area at Indian Gap in the Great Smoky Mountains.

Water samples were collected several times during a 2-year period from site 1, a small stream immediately below plot II and within the sprayed area. Site 2 was established below the treated area in a small stream which had been formed by drainage from the treated area. Because the streams were very small, the entire cross section of the stream was sampled. On each sampling date, three 3.8-liter samples were taken from each site. The water was stored at 3°-5°C in brown glass jugs. Samples were usually transported to the laboratory on the same day and extracted on the following day. Water was not filtered before extraction.

Grab samples of sediment were taken from the same sites. Each sample was a composite of sediment from five locations 2 to 5 cm deep along an 8-m length of the stream. Field personnel took three samples at each sampling site.

Small mammals were caught periodically in snap-traps within each plot and outside the treated area during a 3-year period after spraying. During trapping periods, trap lines were run at 12-hour intervals. The type and number of animals were limited; therefore, all trapped animals were sacrificed and analyzed for BHC residues. All samples except water were stored at −18°C until analysis.

The extraction method for water was identical to that used by Bradley et al. (*12*). Soil and sediment were air-dried and passed through a No. 18 sieve. Subsamples of 100 g each were extracted by a procedure adapted from one used in the National Soils Monitoring Program (*13,14*).

Forest litter samples were extracted by the aqueous-acetonitrile method for low-moisture and low-fat samples (*15*).

Animals of the same species which weighed less than 40 g were grouped by areas. Animals weighing more than 40 g were analyzed separately. The entire animal, including skin and digestive tract, was analyzed. A frozen animal sample was chopped in a Hobart food chopper with sufficient anhydrous sodium sulfate to absorb the moisture. The entire sample was transferred to 500-ml centrifuge bottles. Each bottle was extracted three times with petroleum ether (200, 100, and 100 ml, respectively). The bottles were centrifuged at 1500 rpm for 5 minutes and the solvent layer was decanted through anhydrous sodium sulfate. Extracts were combined, and the solvent was evaporated with a stream of dry air. Fatty materials remained.

A 3-g aliquot of animal fat was subjected to the standard acetonitrile partitioning procedure (*15*). Extracts from the acetonitrile partitioning of the fat and the litter extract were chromatographed on an activated florisil column; BHC was eluted with 6 percent diethyl ether in petroleum ether (*15*). Water, soil, and sediment extracts did not require cleanup before gas chromatography.

The alpha, beta, and gamma isomers of BHC were determined with a model MT-220 gas chromatograph equipped with a ^{63}Ni electron-capture detector. A 183-cm-by-0.3-cm, U-shaped, glass column packed with 6 percent QF-1 and 4 percent SE-30 on Gas Chrom Q (60/80 mesh) was used with a nitrogen flow rate of 100 cc/min. Column, injection port, and detector temperatures were 175°, 220°, and 250°C, respectively.

All gas chromatographic measurements were made by the peak height method; amounts present were calculated against standards run daily. Recoveries of added

amounts of BHC from soil, fat, litter, and water are in Table 1.

Results and Discussion

Rainfall and temperature data were taken near the experimental area at the same elevation (Table 2). These data show that the weather was typical for the high mountainous area of the Southeastern United States. Sampling sites received little or no direct sun and the area was cool and damp, even in summer months.

The experimental area had been sprayed with BHC at approximately 11 kg/ha. in 1963. Litter and soil samples collected before the 1967 application contained BHC residues of 67 and 17 ppm, respectively (Tables 3,4).

Initially, the forest litter contained the highest level of BHC. The concentration in the litter averaged 585 ppm about 1 month after application; residues decreased gradually over the next 5 years to 27 ppm.

Because pretreatment BHC levels in soil averaged 17 ppm, the initial deposit in the surface soil from the 1967 spraying was 31 minus 17 ppm, or about 14 ppm; surface soil was usually but not always beneath a layer of litter. BHC residues in soil increased to a peak of 58 ppm 1.5 years after application. Thereafter, the concentration slowly declined to 32 ppm 3.5 years later.

Although no specific weights of soil and litter layers were recorded, authors do know that the 15-cm soil layer weighs several times as much as the overlying litter. Therefore, the increase of BHC concentration in the soil from about 31 ppm at the first sampling date after application to the peak concentration of 58 ppm 1.5 years later may account for 50 percent or more of the BHC lost from the litter (585 ppm to 134 ppm) during this same period. Extended persistence of the insecticide in this soil probably can be attributed to conditions unfavorable to microbial activity: highly acid soils, cool soil temperatures most of the time, and low intensity of solar radiation within the forest area (*3,10*).

The present data on persistence of BHC in soil are in general agreement with those of other authors who showed that BHC persisted at least 14 years in soils of a climate more moderate than the mountains of North Carolina (*3,5*).

Residues in the water from site 2, which drains the entire watershed, were usually below the limit of detection (0.06 ppb:Table 5). On August 30, 1967, after several heavy rains between August 19 and August 27 totaling 18 cm, the stream flow was above normal. Insecticide spraying had been completed by August 30, and the BHC level in samples from the stream averaged 6.3 ppb. April 16, 1969, was the only other sampling date on which there was an appreciable amount of BHC in the stream below the sprayed area. This sampling was made during runoff of melting snow. Water collected from the small stream within the treated area usually contained low concentrations of BHC (Table 5).

Sediment from site 1, the stream within the treated area, contained residues from 0.49 to 3.17 ppm (Table 6). Residues in sediment from site 2, the stream below the treated area, ranged from <0.03 to 0.21 ppm.

Total residues in the fat of animals taken from treated areas ranged from 0.6 to 106 ppm, and those in fat of animals from outside the treated area ranged from 0.1 to 176 ppm (Table 7). There was no relation between BHC residues in animals and the trapping area (treated versus untreated); therefore, no animals were sampled after 1969. Most animals sampled in 1967 and 1968

TABLE 1. *Recoveries of BHC isomers from animal fat, forest litter, soil or sediment, and water*

TYPE SAMPLE	BHC ISOMER	NO. SAMPLES	AMOUNT ADDED [1]	AVERAGE RECOVERY, %	RECOVERY RANGE, %	SENSITIVITY LEVEL [1]
Animal fat	Alpha	8	0.1-100	92	87- 95	0.02
	Beta	8	0.3-300	92	82- 97	0.06
	Gamma	8	0.1-100	91	84- 95	0.02
Forest litter	Alpha	15	0.2-40.0	99	80-112	0.01
	Beta	15	0.2-200	98	81-110	0.01
	Gamma	15	0.2-200	103	82-142	0.01
Soil or sediment	Alpha	18	0.05-10.0	98	82-127	0.01
	Beta	18	0.05-20.0	94	54-136	0.01
	Gamma	18	0.05-20.0	97	78-126	0.01
Water	Alpha	8	0.02-1.0	80	65- 91	0.02
	Beta	8	0.02-5.6	93	73-148	0.02
	Gamma	14	0.02-2.0	82	70- 90	0.02

NOTE: BHC was added immediately before extraction.
[1] Units are ppm for animal fat, forest litter, soil, and sediment, and ppb for water.

and all those sampled in 1969 were from outside the treated area, although they normally travel across treated and some untreated portions of the area. Attempts to trap animals in the treated area were unsuccessful, and no dead animals were found during routine searches.

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TABLE 2. *Climatological data near BHC experimental area, North Carolina—1967-72*

Year	Month	Temperature High (°C)	Temperature Low (°C)	Temperature Avg (°C)	Rainfall, cm
1967	June	21.1	2.8	11.7	28.3
	July	20.0	3.3	11.7	22.7
	Aug.	20.0	2.2	11.1	19.9
	Sept.	18.9	—6.1	6.1	11.2
	Oct.	18.9	—5.6	6.7	12.1
	Nov.	13.3	—17.2	—2.2	10.5
	Dec.	14.4	—16.7	—1.1	19.3
1968	Jan.	12.8	—18.9	—3.3	19.4
	Feb.	10.0	—21.1	—5.6	1.8
	Mar.	17.2	—17.8	—0.6	21.8
	Apr.	20.0	—3.3	8.3	17.0
	May	20.0	—3.3	8.3	14.1
	June	24.4	3.3	13.9	31.7
	July	21.1	2.2	11.7	10.3
	Aug.	22.8	5.6	13.9	12.6
	Sept.	20.0	3.3	11.7	9.4
	Oct.	17.8	—8.9	4.4	32.6
	Nov.	18.3	—14.4	1.7	12.0
	Dec.	18.3	—14.4	1.7	12.7
1969	Jan.	8.9	—21.7	—6.7	13.1
	Feb.	7.8	—17.8	—5.0	22.3
	Mar.	7.8	—17.8	—5.0	19.4
	Apr.	20.0	—7.8	6.1	20.3
	May	21.1	—5.6	7.8	15.3
	June	23.3	0	11.7	19.9
	July	22.8	10.0	16.1	18.0
	Aug.	20.0	6.1	12.8	18.2
	Sept.	17.8	3.3	10.6	12.8
	Oct.	21.1	—7.8	6.7	15.1
	Nov.	13.3	—20.0	—3.3	24.3
	Dec.	8.9	—14.4	—2.8	23.1
1970	Jan.	8.9	—31.1	—11.1	9.6
	Feb.	14.4	—24.4	—5.0	12.0
	Mar.	12.2	—17.8	—2.8	13.0
	Apr.	19.4	—11.1	3.9	15.7
	May	21.1	—1.1	10.0	8.7
	June	21.1	3.3	12.2	12.3
	July	21.1	5.6	13.3	20.9
	Aug.	21.1	7.8	14.4	21.8
	Sept.	22.8	—2.2	10.0	6.3
	Oct.	16.1	—4.4	5.6	45.6
	Nov.	12.2	—22.8	—5.6	14.8
	Dec.	10.0	—20.0	—5.0	10.3
1971	Jan.	10.0	—18.9	—4.4	16.8
	Feb.	11.7	—18.9	—3.9	19.8
	Mar.	13.3	—18.9	—2.8	16.3
	Apr.	17.8	—7.8	5.0	11.5
	May	20.0	—8.9	5.6	14.1
	June	21.1	7.8	14.4	10.9
	July	21.1	4.4	12.8	22.2
	Aug.	20.0	7.2	13.3	24.0
	Sept.	20.0	5.6	12.8	14.2
	Oct.	18.9	—3.3	7.8	34.9
	Nov.	15.6	—14.4	0.6	19.7
	Dec.	15.6	—14.4	0.6	19.6
1972	Jan.	12.2	—12.2	0	21.7
	Feb.	15.0	—17.8	—1.7	12.9
	Mar.	14.4	—14.4	0	20.5
	Apr.	20.0	—12.2	3.9	11.3
	May	15.6	—2.2	6.7	24.9
	June	18.9	—2.2	8.3	33.4
	July	21.1	3.3	12.2	20.2
	Aug.	20.0	8.9	14.4	4.9

TABLE 3. *BHC residues in forest litter, North Carolina—1967-72*

Sampling date	Plot	BHC isomer Alpha (ppm)	BHC isomer Beta (ppm)	BHC isomer Gamma (ppm)	Total (ppm)
5/18/67	Check [1]	0.04	0.01	0.01	0.06
5/19/67	1 [2]	4.68	19.1	9.00	32.78
	2	11.9	75.9	26.4	114.2
	3	4.78	40.4	10.1	55.37
7/7/67	1	146	298	252	696.0
	2	88.6	188	158	434.6
6/30/67	3	119	302	205	626.0
8/2/67	1	98.2	254	188	540.2
	2	71.2	226	142	439.2
	3	46.0	152	91.4	289.4
9/1/67	1	66.5	256	142	464.5
	2	44.4	148	95.4	287.8
	3	60.0	210	126	396.0
10/31/67	1	60.3	262	130	452.3
	2	35.8	150	80.8	266.6
	3	47.4	238	106	391.4
5/23/68	1	74.9	236	188	498.9
	2	44.5	170	101	315.5
	3	73.5	286	178	537.5
6/25/68	1	42.6	182	102	326.6
	2	33.8	134	79.4	247.2
	3	22.8	98.7	48	169.5
11/6/68	1	17.2	91.5	41	149.7
	2	13.5	56.2	32.1	101.8
	3	19.2	83.4	47.9	150.5
8/20/69 [3]	1	19.6	73.0	41.1	133.6
	2	17.8	58.2	33	109.0
	3	18.7	71.0	40.6	130.3
10/29/70	1	6.46	11.2	39.4	57.06
	2	4.86	9.22	30.4	44.48
	3	8.33	19.2	58	85.53
7/19/72	1	4.07	20.3	7.38	31.75
	2	2.14	10	3.88	16.02
	3	3.62	22.0	7.20	32.82

NOTE: Residues expressed are as received.
All values are averages of two samples except for those from check plot.

[1] Average of three samples from Indian Gap, Great Smoky Mountains.

[2] Samples collected May 19, 1967, are pretreatment samples from experimental area.

[3] Average of three samples.

TABLE 4. *BHC residues in soil, North Carolina—1967-72*

Sampling date	Plot	BHC isomer			Total (ppm)
		Alpha (ppm)	Beta (ppm)	Gamma (ppm)	
5/18/67	Check [1]	0.01	0.01	0.08	0.10
5/19/67	1 [2]	0.81	3.36	1.82	5.99
	2	1.50	6.36	3.46	11.32
	3	4.72	10.6	17.2	32.52
7/6/67	1	4.15	14.2	8.34	26.69
	2	3.48	13.4	6.72	23.60
6/30/67	3	4.69	27.6	9.79	42.08
8/2/67	1	1.44	4.56	2.80	8.80
	2	4.44	12.9	7.59	24.93
	3	2.89	12.1	6.14	21.12
9/1/67	1	4.82	13.3	8.44	26.56
	2	5.54	16.0	10.8	32.34
	3	5.26	18.2	10.7	34.16
10/31/67	1	4.03	16.4	9.33	29.76
	2	4.18	14.8	7.77	26.75
	3	4.72	17.6	9.40	31.72
5/23/68	1	5.21	15.7	10.6	31.51
	2	3.77	14.6	8.68	27.05
	3	7.34	30.6	16.0	53.94
6/25/68	1	4.50	14.7	8.50	27.70
	2	2.53	10.9	5.92	19.35
	3	6.66	25.4	15.2	19.35
11/6/68	1	10.3	34.6	21.9	66.80
	2	7.58	26.6	21.8	55.98
	3	7.42	25.8	17.6	50.82
8/20/69 [3]	1	8.68	17.3	17.7	43.68
	2	3.84	9.43	8.03	21.30
	3	6.09	12.4	13.7	32.19
10/29/70	1	3.72	13.0	11.7	28.42
	2	3.22	12.0	11.7	26.92
	3	8.16	22.6	24.1	54.86
7/19/72	1	2.74	11.1	6.16	20.00
	2	3.42	20.2	7.27	30.89
	3	5.30	28.6	11.7	45.60

NOTE: Residues expressed are oven-dry weight.
Values are the average of two samples except as indicated.
[1] Average of three samples from Indian Gap, Great Smoky Mountains.
[2] Samples collected May 19, 1967, are pretreatment samples from experimental area.
[3] Average of three samples.

TABLE 5. *BHC residues in water within and below treated area, North Carolina—1967-69*

Sampling date	Site [1]	BHC isomer			Total (ppb)
		Alpha (ppb)	Beta (ppb)	Gamma (ppb)	
5/18/67	Control	<0.02	<0.02	<0.02	<0.06
5/19/67	1 [2]	0.04	<0.02	0.12	0.17
	2	<0.02	<0.02	0.02	<0.06
7/ 6/67	1	1.14	1.13	2.63	4.90
	2	<0.02	<0.02	0.03	<0.06
7/ 7/67	2 [3]	<0.02	<0.02	0.03	<0.06
7/29/67	2 [3]	<0.02	<0.02	<0.02	<0.06
8/ 1/67	2 [3]	<0.02	<0.02	<0.02	<0.06
8/ 2/67	1	0.86	1.82	2.11	4.79
	2	<0.02	<0.02	<0.02	<0.06
8/22/67	1	3.57	4.18	8.67	16.42
	2	<0.02	0.04	0.03	0.08
8/30/67	1	<0.02	<0.02	<0.02	<0.06
	2	1.15	2.42	2.70	6.27
10/31/67	1	0.06	0.10	0.16	0.32
	2	<0.02	<0.02	<0.02	<0.06
5/23/68	1	0.21	2.75	1.71	4.67
	2	<0.02	<0.02	<0.02	<0.06
6/25/68	1	0.75	5.04	2.28	8.07
	2	<0.02	0.03	<0.02	<0.06
11/ 6/68	2	<0.02	<0.02	<0.02	<0.06
4/16/69	1	0.92	2.43	2.18	5.52
	2	0.19	0.28	0.27	0.74

NOTE: Values are averages of three samples analyzed separately except as indicated.
[1] Site 1 is within treated area; site 2 is below.
[2] Only two samples analyzed.
[3] Values are averages of three samples collected during a rainstorm on each date.

TABLE 6. *BHC residues in sediment within and below treated area, North Carolina—1967-69*

Sampling date	Site	BHC isomer			Total (ppm)
		Alpha (ppm)	Beta (ppm)	Gamma (ppm)	
7/ 6/67	1	0.34	2.00	0.83	3.17
	2	0.01	0.02	0.10	0.13
8/ 2/67	1	0.07	0.71	0.16	0.94
	2	<0.01	0.01	<0.01	<0.03
9/ 1/67	1	0.07	0.46	0.15	0.68
	2	0.01	0.03	0.01	0.05
10/31/67	2	0.01	0.06	0.01	0.08
5/23/68	1	0.06	0.42	0.16	0.64
	2	0.01	0.18	0.02	0.21
6/25/68	1	0.04	0.34	0.11	0.49
	2	0.02	0.05	0.02	0.09
11/ 6/68 [1]	2	0.02	0.03	<0.01	0.06

NOTE: Residues expressed are oven-dry weight. Values are the average of three samples except as indicated.
[1] Average of two samples.

TABLE 7. *BHC residues in animal fat, North Carolina—1967-69*

Sampling year	Location	Number and type of animal [1]	Lipid, %	BHC isomers			Total (ppm)
				Alpha (ppm)	Beta (ppm)	Gamma (ppm)	
1967	Plot I	3 Deer mice	12.6	0.70	1.20	0.30	2.20
		1 Little brown myotis bat	1.4	0.63	2.93	0.86	4.42
		3 Deer mice	7.9	36.4	42.2	27.5	106.1
		1 Boreal redback vole	5.8	0.37	0.55	0.27	1.19
	Plot II	1 Deer mouse	9.7	3.06	6.01	4.85	13.92
		1 Boreal redback vole	7.6	0.15	0.19	0.25	0.59
	Plot III	1 Deer mouse	7.4	0.32	0.60	0.57	1.49
		1 Boreal redback vole	3.3	2.51	3.96	2.58	9.05
	Outside treated area	2 Deer mice	7.0	0.38	0.52	0.24	1.14
		1 Deer mouse	4.1	16.9	17.6	4.58	39.08
		1 Dear mouse	5.9	0.20	0.78	0.31	1.29
		3 Deer mice	8.2	0.51	0.67	0.43	1.61
		1 Boreal redback vole	3.3	0.11	0.45	0.20	0.76
		1 Boreal redback vole	2.9	0.09	0.34	0.18	0.61
		1 Boreal redback vole	5.7	0.07	0.27	0.12	0.46
		1 Boreal redback vole	6.3	0.11	0.18	0.28	0.57
		2 Boreal redback voles	6.3	61.4	29.6	56.8	147.8
		1 Boreal redback vole	2.9	36.0	127.	12.5	175.5
		1 Shorttail shrew	2.4	0.27	0.84	0.51	1.62
		3 Shorttail shrews	8.2	11.9	33.6	13.3	58.8
		1 Woodland jumping mouse	3.0	0.23	0.47	0.35	1.05
		1 Woodland jumping mouse	1.4	5.23	24.2	7.10	36.53
1968	Plot I	3 Deer mice	13.8	1.55	2.98	0.97	5.50
		3 Deer mice	16.2	0.98	1.38	0.71	3.07
		3 Deer mice	19.8	0.69	0.83	0.38	1.90
		1 Boreal redback vole	8.8	9.33	2.94	3.00	15.27
		1 Boreal redback vole	33.4	1.25	1.54	0.69	3.48
	Outside treated area	3 Deer mice	10.2	1.24	1.67	0.90	3.81
		3 Deer mice	13.2	0.10	0.16	0.08	0.34
		3 Deer mice	11.3	0.20	0.25	0.11	0.56
		1 Boreal redback vole	8.1	0.03	0.11	0.03	0.17
		1 Boreal redback vole	15.3	0.02	0.07	0.02	0.11
		1 Boreal redback vole	3.5	3.46	3.91	1.29	8.66
		1 Norway rat	6.5	0.45	0.14	0.20	0.79
		1 Woodchuck	4.8	0.29	0.41	0.19	0.89
1969	Outside treated area	2 Deer mice	11.7	0.09	0.34	0.21	0.64
		2 Deer mice	13.4	<0.02	<0.06	0.02	<0.10
		1 Boreal redback vole	13.0	0.02	0.06	0.02	0.10
		1 Boreal redback vole	19.2	0.05	0.24	0.02	0.31

NOTE: Residues expressed as extractable lipids.

[1] Deer mouse, *Peromyscus maniculatus*; little brown bat, *Myotis lucifugus*; Boreal redback vole, *Clethrionomys gapperi*; short-tailed shrew, *Blarina brevicauda;* woodland jumping mouse, *Napaeozapus insignis;* Norway rat, *Rattus norvegicus;* and woodchuck, *Marmota monax.*

Contribution of Household Dust to the Human Exposure to Pesticides[1]

Herbert G. Starr, Jr.,[2] Franklin D. Aldrich,[3] William D. McDougall III,[4]
and Laurence M. Mounce [2]

ABSTRACT

Preliminary analysis of environmental contributions to pesticide body burden revealed household dust as a major reservoir of pesticides in the environment. A year-long monthly study of the households of pesticide-exposed persons and control households in Weld County, Colo., in 1968 revealed appreciable levels of selected chlorinated pesticides in the exposed group. Exposed subjects varied from entire farm families with high agricultural use of pesticides to households with at least one member who formulated pesticides, either as an employee of a local plant or as a professional applicator who mixed and loaded pesticides for commercial use. In the overall data no quantitative relationships were demonstrated between pesticide levels in household dust and pesticide levels in blood, although circumstantial data from individual households indicate that a certain connection does exist. No correlation could be shown between levels of p,p'-DDT and p,p'-DDE in household dust. Pesticide levels in the dust indicate a probable influence on body burden contributing to total environmental exposure of the individual to pesticides.

Introduction

Pesticide body burden in the general population can be augmented by dust, air (*1*), water, food, and the nature of the home and working environments. Differences in pesticide levels distinguishing the general population from a pesticide-exposed population may then be attributed to occupational exposure and to pesticides carried into homes on clothing and other items. Surveys of pesticide content in food, soil, and water have been conducted for many years without attempting to relate levels found to pesticide body burden. The present study was initiated to determine the relationship between pesticide levels in household dust and pesticide levels in human blood sera in Weld County, Colo., which has a high annual usage of pesticide chemicals because it is primarily an agricultural area.

Preliminary analyses of soil, water, and household dusts conducted in 1966 revealed pesticide levels to be higher in household dusts than in soil and water, and indicated some correlation between DDT levels in dust and DDT and DDE levels in sera of householders (*2*). In order to minimize the variables associated with different sampling times and seasonal peaks in pesticide use, the study was conducted for 1 year. From multiple regression analyses and available data, the following hypothesis was formed: a definable relationship exists between blood serum chlorinated hydrocarbon pesticide levels and household dust levels.

Reviews of published literature revealed no attempt to measure pesticide levels in household dusts. Modifications of other soil assay methods were incorporated in the analytical method for this study to provide sensitivity and specificity needed for accurate analyses of pesticides in household dusts.

Storm dust fall has been analyzed (*3*) for organochlorine pesticide levels using microcoulometric/gas-liquid chromatography (MC/GLC) in the quantitative step. Pesticide levels in soils have been studied by many researchers who have designed a variety of soil extraction and cleanup techniques for adaptation to current needs (*4-6*). This paper attempts to report results obtained from a year's study of pesticide levels in both household dusts and blood sera, and, when possible, to demonstrate the relationship of pesticide levels between these two substrates.

[1] Research supported by contract PH-86-65-62 with the Division of Community Studies, Atlanta, Ga. Formerly part of Food and Drug Administration, U.S. Department of Health, Education, and Welfare. Presently under Technical Services Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C.

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Analytical Methods

HOUSEHOLD DUST

Household dust from a month's sweepings was collected in disposable vacuum cleaner bags and sifted. Particles passing through a Number 60 mesh screen were taken for analysis. One gram of air-dried dust was soxhlet-extracted with 1:1 hexane:acetone and column-chromatographed on deactivated aluminum oxide using hexane as the eluent to remove waxes and other extractables. Final separation of chlorinated hydrocarbon pesticides was obtained by florisil column chromatography. Final concentrations of household dust extracts were 2.0 μg/μl for analysis by electron-capture/gas-liquid chromatography (EC/GLC) and 1.0 mg/μl for analysis by MC/GLC. An arbitrary sensitivity limit of 1.50 parts per million (ppm) was established so that all residues could be qualitatively confirmed by MC/GLC. Organochlorine insecticide recovery data for fortified household dust screenings are given in Table 1. All recoveries were within acceptable analytical ranges. Relatively high standard deviations may have been caused by difficulty in obtaining uniform fortification rather than by the method.

TABLE 1. *Recovery of pesticides from fortified housedust samples, Colorado—1968*

Compound	No. Samples	Mean Recovery, %	Standard Deviation [1]
Lindane	12	87.3	6.99
Heptachlor Epoxide	12	80.6	12.86
Dieldrin	11	86.3	12.89
p,p'-DDT	9	87.8	20.20
p,p'-DDE	12	82.9	6.35
p,p'-DDD	12	90.0	9.72

[1] Relatively high standard deviations possibly caused by difficulty in obtaining uniform fortification rather than by method.

BLOOD

Blood samples were collected in 10-ml Vacutainer tubes containing no anticoagulant. After clotting, 2.0 ml serum was extracted with hexane using the method of Dale, Curley, and Cueto (*7*). Samples were analyzed by EC/GLC with an arbitrary lower sensitivity limit of 5 parts per billion (ppb).

GAS CHROMATOGRAPHY

All analyses were performed on Micro-Tek 220 gas chromatographs equipped with H^3 electron-capture detectors. Household dust residues were qualitatively verified with a chlorine-specific microcoulometric detector. Gas-chromatographic liquid phases used for this study were coated on acid-washed and silanized support. Phases were 1.5 percent OV-17/1.95 percent QF-1 (column A); 4 percent SE-30/6 percent QF-1 (column B); and 3.5 percent QF-1/6.5 percent DC-200 (column C). Operating conditions were established to yield optimum separation with reasonable retention time for the latest eluting compounds.

Experimental Design

During the 12 months of 1968, household dust samples were collected from 28 households; blood samples were obtained from 28 heads of households and 27 spouses on the same sampling dates. Household dust samples consisted of the entire month's vacuum sweepings with at least two sweepings a week. Homes were selected on the following bases: location in Weld County, past cooperation with the program, pobability and consistency of continuing participation, and willingness of the family to participate in a long-term study requiring monthly sampling. The sampling group consisted of 16 urban control households, 4 farm households, and 8 households with at least one member who was a pesticide formulator.

Control participants had no known occupational exposure to pesticides and only minimal home-use exposure. Farm participants were selected from those with highest agricultural usage of pesticides among participants in the Colorado Community Study on Pesticides (formerly under Food and Drug Administration, U.S. Department of Health, Education, and Welfare; currently under Technical Services Division, U.S. Environmental Protection Agency—Office of Pesticide Programs). The formulator group ranged from those employed in local pesticide formulating plants to professional applicators who mixed and loaded pesticides for commercial use.

Results and Discussion

Table 2, a summary of each study group and substrate, lists total number of samples, frequency of occurrence, mean of values above the minimum sensitivity limits, and range of values for all pesticides detected during the 1-year sampling period. Gas-chromatographic column C was utilized to analyze all samples through June 1968 and did not provide adequate resolution or sensitivity for determining chlordane in blood. Samples analyzed after June were analyzed on gas-chromatographic columns A and B utilizing a modification of the florisil column elution of two fractions improving resolution of pesticides occurring in household dust samples.

Because of widespread usage of DDT over the past 25 years, residues occurring most frequently in blood were *p,p'*-DDT and its metabolite, *p,p'*-DDE; in household dust, the most common residue was *p,p'*-DDT. Also occurring frequently in household dust was *o,p'*-DDT, the technical isomer of *p,p'*-DDT. It did not occur in the blood samples because it is rapidly metabolized and excreted. Table 3 is a statistical evaluation of the 12-month average of *p,p'*-DDT and *p,p'*-DDE in blood, and *p,p'*-DDT in household dust.

Correlation coefficients based on all 28 households showed a statistically significant positive correlation. It was observed, however, through inspection of data from each household that one household consistently yielded

TABLE 2. *Pesticide residues in human blood sera and household dust in 28 households, Weld County, Colo.—1968*

		Control Group		Farmer		Formulator		Control Group	Farmer	Formulator
No. Samples		M 187	F 171	M 46	F 47	M 88	F 87	182	45	95
		Blood Residues, ppb						Housedust Residues, ppm		
p,p'-DDT	F	94	96	36	11	84	56	159	42	95
	R	5-14	5-68	5-19	5-6	5-68	5-21	1.56-35.44	1.60-37.80	2.34-226.15
	$\bar{X}$	7.7	8.1	9.5	5.4	17.9	9.4	6.90	8.87	30.66
p,p'-DDE	F	187	170	46	47	88	87	4	9	46
	R	5-95	5-105	14-116	7-28	16-209	9-82	1.50-12.28	1.63-7.55	1.50-17.10
	$\bar{X}$	28.3	24.3	48.4	17.0	54.8	30.1	4.37	3.28	4.83
o,p'-DDT	F							56	16	55
	R							1.52-10.20	1.62-5.31	1.63-21.99
	$\bar{X}$							2.67	2.91	6.31
p,p'-DDD	F			1				2	8	9
	R			5				1.73-1.81	2.22-19.21	1.59-7.03
	$\bar{X}$			5.0				1.77	6.11	2.95
Methoxychlor	F							78	21	56
	R							1.53-28.57	1.58-102.90	1.92-144.44
	$\bar{X}$							6.09	14.93	18.24
Lindane	F					11 [1]	1		3	43
	R					7-23	5		1.75-2.27	1.54-13.72
	$\bar{X}$					16.8	5.0		2.05	5.85
β-BHC	F	15	7	19	10	25	11			
	R	5-8	9-15	5-27	5-12	6-59	6-22			
	$\bar{X}$	5.9	10.9	12.4	8.8	18.7	9.5			
Chlordane	F					6 [1]		45	10	77
	R					60-233		1.79-41.36	1.92-10.72	2.15-135.78
	$\bar{X}$					151		7.59	5.79	23.11
Dieldrin	F	1				47	13 [2]	25	15	59
	R	5				5-140	5-14	1.59-10.21	1.59-10.63	1.59-40.42
	$\bar{X}$	5.0				43.5	10.1	2.94	4.42	8.92
Heptachlor Epoxide	F					21				
	R					7-22				
	$\bar{X}$					10.9				
Endrin	F									27
	R									1.50-27.73
	$\bar{X}$									7.14
Dacthal	F							14	22	19
	R							1.53-32.59	1.90-59.71	1.55-41.91
	$\bar{X}$							7.11	18.50	7.28

NOTE: F = Frequency of values above minimum sensitivity limit.
R = Range of values above minimum sensitivity limit.
$\bar{X}$ = Mean of values above minimum sensitivity limit.
[1] One participant only.
[2] Eleven values from one participant.

residue levels several magnitudes above the means of the other households. By omitting data from the one household, the correlations remained positive but lost any statistical significance, as demonstrated in Table 3. Hence the contribution of the one household to the significant correlation was disproportionate.

Mean *p,p'*-DDT in the one household's dust was 131.14 ppm; in the other seven formulator households the mean was 16.13 ppm. Mean *p,p'*-DDT and *p,p'*-DDE in blood of the male occupant of the household with highest residues were 46.8 ppb and 143.3 ppb, respectively, whereas male occupants of the other seven households had means of 12.3 ppb and 40.9 ppb, respectively. Although these data suggest a trend, they do not prove statistical correlations between *p,p'*-DDT levels in household dust and *p,p'*-DDT/*p,p'*-DDE levels in human blood.

Interpretations of frequencies and ranges of some pesticides listed in Table 2 can be made. The relatively high frequency of chlordane occurrence in control household dust was caused by its use in many common household insecticides. Occurrence of the herbicide Dacthal in farm household dusts was caused by its use on certain produce croplands in the Weld County area. Dacthal occurred in lower levels among formulators and control groups, probably because these individuals had no history of occupational exposure to this pesticide.

Three values requiring further explanation are chlordane and lindane in the blood of a male formulator and dieldrin in the blood of his wife. These unusual findings of chlordane and lindane in blood and a related medical problem in this male participant have been published previously (*8*). Mean chlordane and lindane levels in the dust from this home were 76.46 ppm and 10.90 ppm, respectively; in the other seven formulators' homes mean values were 10.18 ppm and 1.58 ppm, respec-

tively. Because neither chlordane nor lindane residues are commonly found in human blood except in acute exposure situations, this observation seems to present circumstantial evidence relating household dust residues to body burden. The mean dieldrin level in this subject's household dust was 28.02 ppm compared to 2.29 ppm for the other seven formulators' households. As a result, 11 of 13 occurrences of dieldrin in the blood of formulators' wives were obtained from this participant. This case provides further circumstantial evidence relating household residue levels to blood residue levels because this woman had no history of occupational exposure to dieldrin.

TABLE 3. *Correlation coefficients for levels of DDT in household dust with levels of DDT and DDE in human blood, Colorado—1968*

		Household Dust [1] p,p'-DDT	
		28 Households [2]	27 Households
Blood	p,p'-DDT	0.81	0.25
	p,p'-DDE	0.75	0.11

NOTE: Product-moment correlation coefficients.
Coefficients represent 12-month average.

[1] Coefficients first calculated for 28 households, then recalculated omitting one household with extremely high residues which overly influenced correlations; data could not be considered statistically significant.

[2] Values significant at the 0.01 level.

Conclusions

Data from this study have shown no quantitative relationships between pesticide levels in household dust and pesticide levels in blood. Circumstantial data from individual households indicate that at least some relationships do exist; however, no statistical significance can be demonstrated. It is evident from the current literature as well as data presented here that a simplified approach to the problem of relating pesticide body burden to a specific type of exposure, either environmental or dietary, is difficult because of the many variables in this complex problem.

Acknowledgments

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BRIEF

A Nomograph for the Conversion of 2,4-D Ester Concentrations in Air from μg/m³ to ppb$_v$ and Vice Versa[1]

Raj Grover and Barry McCashin

ABSTRACT

A nomograph for the conversion of 2,4-D (2,4-Dichlorophenoxyacetic acid) ester concentrations from μg/m³ to ppb$_v$ and vice versa has been prepared to provide a simple and direct means for such conversions. Results obtained are shown to be comparable to those calculated.

Introduction

Data on direct measurements of pesticide residue levels in the atmosphere are being reported in increasing numbers. Air pollution aspects (*1*) and hazards from off-target drift of agriculturally generated pollutants (*2-4*), especially pesticides, have recently been reviewed. Measurements of pesticide levels in the atmosphere are generally expressed in weight/volume (w/v) units, i.e., nanogram or microgram of pesticide per cubic meter of air (ng or μg/m³). However, the cubic meter unit is dependent on ambient conditions of temperature and pressure which should be designated. When dealing with gaseous pollutants in the atmosphere, it is often desirable to denote concentrations in relative volumes of w/v units, i.e., parts of pesticide per million or billion parts of air (ppm$_v$ or ppb$_v$). Because the ratio of volumes is unaffected by fluctuations in temperature and pressure during sampling, ppb$_v$ or ppm$_v$ units do not change as volumes change.

Procedures for conversion of concentrations from μg/m³ to ppb$_v$ or ppm$_v$ have been discussed in recent texts on air sampling methodology (*5,6*). However, nomographs provide a simple and direct means for such conversions and have been developed for a variety of gaseous compounds (*7*).

Concentrations of any gaseous compound in the air can be converted from μg/m³ to ppb$_v$ using the following expression:

$$y\left[\frac{\mu g}{m^3}\right] \times \frac{1}{w \times 10^6}\left[\frac{\text{g mole}}{\mu g}\right] \times v\left[\frac{m^3}{\text{g mole vol}}\right] \times 10^9 \left[\frac{\text{parts}}{\text{billion parts}}\right] = z\ [\text{ppb}_v]$$

where y = concentration of the residue in μg/m³,
w = gram molar weight of the compound,
v = gram molar volume of air in m³,
and z = concentration of residue in ppb.

Example 1. Convert 40 μg/m³ methyl ester of 2,4-D sampled at 20°C and 1 atm pressure (STP) to parts per billion (ppb$_v$).

$$y = 40\left[\frac{\mu g}{m^3}\right]$$

$$w = 235.08\ (\text{g mole wt})$$

$$v = 0.024\left[\frac{m^3}{\text{g mole vol}}\right] \text{ at STP}$$

Substituting the values for y, w, and v in the above expression,

$$40 \times \frac{1}{235.08 \times 10^6} \times 0.024 \times 10^9 = 4.08\ \text{ppb}_v.$$

Similar conversions for other 2,4-D esters were also carried out, using different temperature and pressure conditions (Table 1). In all these conversions, determination of g mole volumes of air in m³ at various pressure and temperature conditions is necessary.

While preparing the nomograph, extremes of pressure and temperature conditions most likely to occur in air

[1] Contribution from the Herbicide Behavior in Environment Section, Research Station, Agriculture Canada, Regina, Saskatchewan, S4P 3A2, Canada.

TABLE 1. *Relative conversion values for various 2,4-D esters, from μg/m³ to ppb$_v$ or vice versa, using calculations and the nomograph*

2,4-D ester	Sampling Conditions			Conversion Values		
				From	To	
	Pressure (mm Hg)	Temp (K)	P/T Factor [1]		Calculation	Nomograph
methyl	733	293	2.50	10 μg/m³	1.1 ppb$_v$	1.1 ppb$_v$
iso-propyl	760	293	2.59	50 μg/m³	4.6 ppb$_v$	4.5 ppb$_v$
n-butyl	700	323	2.17	8 ppb$_v$	77.1 μg/m³	77.0 μg/m³
iso-octyl	800	313	2.56	2 ppb$_v$	26.6 μg/m³	26.3 μg/m³

[1] Pressure/temperature factor.

sampling were selected. These were 20° to 50°C and 700 to 800 mm Hg. Pressure/temperature factors (P/T in mm Hg/K), in the range of 2 to 3, were then plotted against the ppb$_v$ scale on the left side of the log-log paper (Fig. 1). Corresponding ester concentrations were plotted on the right side of the log-log paper in units of μg/m³.

A number of conversions similar to those calculated were read from the nomograph using the procedure shown in Figure 1. The two sets of values, i.e., those calculated and the ones obtained from the nomograph, showed close approximation (Table 1). Conversions beyond the range of the nomograph can also be carried out by simply shifting the decimal point to obtain the appropriate range. The resultant conversion value can then be adjusted to the correct value by placing the decimal point in the reverse direction.

In conclusion, the nomograph greatly facilitates conversion of atmospheric residue levels of 2,4-D esters from μg/m³ to ppb$_v$ and vice versa. It provides a simple and direct means for such conversions while still maintaining a relatively high degree of accuracy.

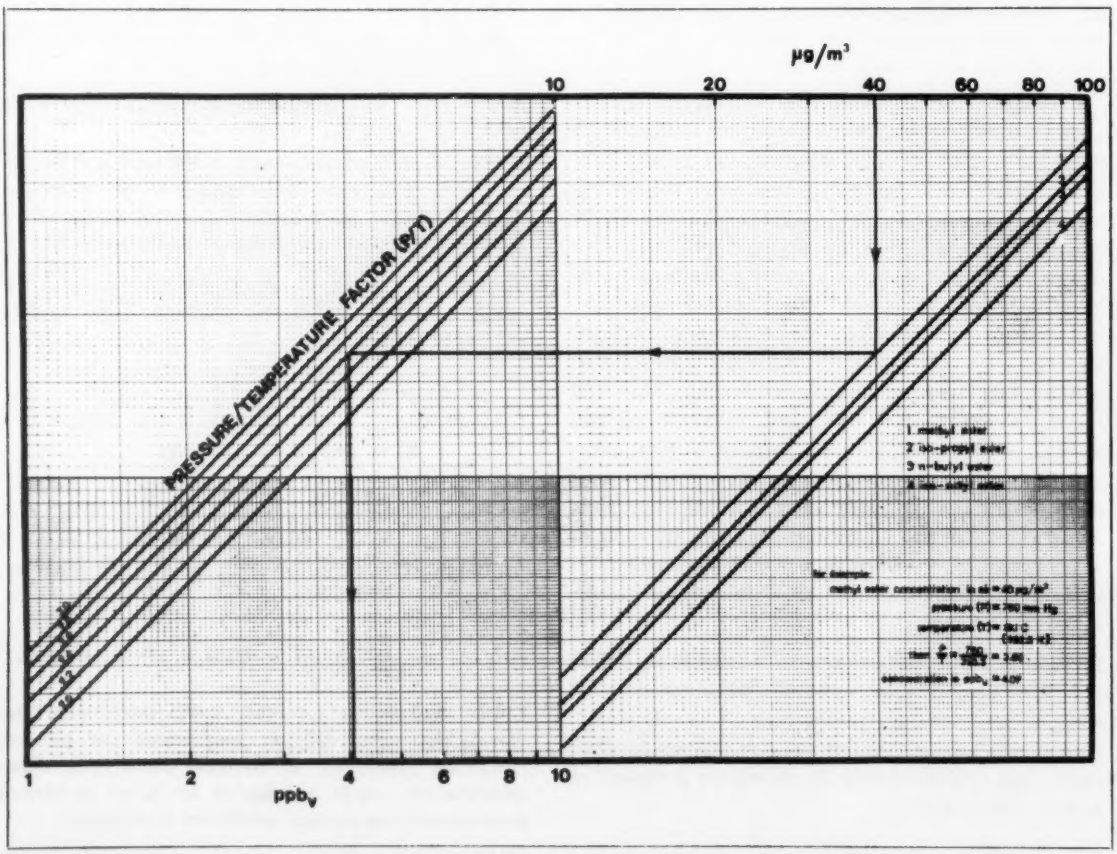

FIGURE 1. *Nomograph for converting concentrations of four esters of 2,4-D from μg/m³ to ppb$_v$ dry air at various pressure/temperature factors (P/T in mm Hg/K)*

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APPENDIX

Chemical Names of Compounds Discussed in This Issue

ALDRIN	Not less than 95% of 1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
BHC (Benzene hexachloride)	1,2,3,4,5,6-Hexachlorocyclohexane (mixture of isomers). Commercial product contains several isomers of which *gamma* is most active as an insecticide.
CHLORDANE	1,2,3,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene. The technical product is a mixture of several compounds, including heptachlor, chlordene, and two isomeric forms of chlordane.
2,4-D	2,4-Dichlorophenoxyacetic acid.
DCPA (Dacthal®)	Dimethyl 2,3,5,6-tetrachloroterephthalate
DDD	See TDE.
DDE	Dichlorodiphenyl dichloro-ethylene. (Degradation product of DDT.) Main component: 1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene *o,p'*-DDE 1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethylene *p,p'*-DDE 1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene
DDT	-Bis (*p*-chlorophenyl) , , -trichloroethane. Numerous isomers in addition to *p,p'*-DDT are possible, and some are present in the commercial product. *o,p'*-DDT [1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane]
DICHLOBENIL	2,6-Dichlorobenzonitrile
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8,-dimethanonaphthalene
ENDRIN	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
FENAC	2,3,6-Trichlorophenylacetic acid
HEPTACHLOR	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-*endo*-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-Heptachloro 2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane
HEXACHLOROBENZENE (HCB)	Perchlorobenzene
LINDANE	Gamma isomer of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) of 99+% purity
METHOXYCHLOR	1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl) ethane
POLYCHLORINATED BIPHENYLS (PCB's)	Mixtures of chlorinated biphenyl compounds having various percentages of chlorine.
TDE (DDD)	2,2-Bis (*p*-chlorophenyl)-1,1-dichloroethane (including isomers and dehydrochlorination products)

Information for Contributors

The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

The publication is distributed principally to scientists and technicians associated with pesticide monitoring, research, and other programs concerned with the fate of pesticides following their application. Additional circulation is maintained for persons with related interests, notably those in the agricultural, chemical manufacturing, and food processing industries; medical and public health workers; and conservationists. Authors are responsible for the accuracy and validity of their data and interpretations, including tables, charts, and references. Accuracy, reliability, and limitations of the sampling and analytical methods employed must be clearly demonstrated through the use of appropriate procedures, such as recovery experiments at appropriate levels, confirmatory tests, internal standards, and inter-laboratory checks. The procedure employed should be referenced or outlined in brief form, and crucial points or modifications should be noted. Check or control samples should be employed where possible, and the sensitivity of the method should be given, particularly when very low levels of pesticides are being reported. Specific note should be made regarding correction of data for percent recoveries.

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——An abstract (not to exceed 200 words) should accompany each manuscript submitted.

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——All copy, including tables and references, should be double spaced, and all pages should be numbered. The first page of the manuscript must contain authors' full names listed under the title, with affiliations, and addresses footnoted below.

——Charts, illustrations, and tables, properly titled, should be appended at the end of the article with a notation in text to show where they should be inserted.

——Charts should be drawn so the numbers and texts will be legible when considerably reduced for publication. All drawings should be done in black ink on plain white paper.

——Photographs should be made on glossy paper. Details should be clear, but size is not important.

——The "number system" should be used for literature citations in the text. List references in the order in which they are cited in the text, giving name of author/s/, year, full title of article, exact name of periodical, volume, and inclusive pages.

The Journal also welcomes "brief" papers reporting monitoring data of a preliminary nature or studies of limited scope. A section entitled *Briefs* will be included, as necessary, to provide space for papers of this type to present timely and informative data. These papers must be limited in length to two journal pages (850 words) and should conform to the format for regular papers accepted by the Journal.

Pesticides ordinarily should be identified by common or generic names approved by national scientific societies. The first reference to a particular pesticide should be followed by the chemical or scientific name in parentheses—assigned in accordance with CHEMICAL ABSTRACTS nomenclature. Structural chemical formulas should be used when appropriate. Published data and information require prior approval by the Editorial Advisory Board; however, endorsement of published information by any specific Federal agency is not intended or to be implied. Authors of accepted manuscripts will receive edited typescripts for approval before type is set. After publication, senior authors will be provided with 100 reprints.

Manuscripts are received and reviewed with the understanding that they previously have not been accepted for technical publication elsewhere. If a paper has been given or is intended for presentation at a meeting, or if a significant portion of its contents has been published or submitted for publication elsewhere, notations of such should be provided.

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